The Catalytic Introduction of Deuterium into Organic Compounds with Special Reference to the Sterols,

with an additional paper on

The Combustion of Sulphur to Sulphur Dioxide.

T H E S I S

submitted for the

Degree of Doctor of Philosophy

at

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by

Samuel James Thomson, B.Sc.

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Preface.

I wish to record my sincere thanks to Dr. James Bell for suggesting the problems, for his guidance in the methods of research and for his constant encouragement and interest.

This work is being prepared for publication in collaboration with Dr. Bell.

My thanks are due to the Department of Scientific and Industrial Research for Maintenance Allowance held during two years of the research.

I am grateful to Mr. Angus Macdonald for conducting the majority of the deuterium assays, and to Miss R.H. Kennaway and Mr. J.M.L. Cameron for the micro-analyses.

Summary.

Bloch and Rittenberg's preparation of deuterio cholesterol by an exchange reaction between the sterol and heavy water in the presence of a platinum catalyst has been repeated. The method has been extended to the preparation of deuterio ergosterol and a comparison made of the extent of the exchange reactions of the two sterols under comparable conditions.

Information on the influence of structure on extent of exchange has been obtained by a study of the exchange reactions of simpler compounds containing structural features of the sterols. The positions of the deuterium atoms have been located in one of these compounds, deuteriocyclohexene.

The compounds undergoing maximum exchange were found to be those undergoing a disproportionation reaction in which the catalyst removed hydrogen from some molecules and donated hydrogen or deuterium to others.

On re-examining the sterol deuteration problem in the light of these experiments on simpler compounds, the catalyst was observed to have the same function, the removal and addition of hydrogen and deuterium atoms to produce the deuterio sterol. In addition the removal of hydrogen atoms from one ring of the sterol molecule and the addition of deuterium or hydrogen atoms to another ring, manifested itself in the migration of carbon-carbon double bonds. Ergosterol Bl has

been isolated in ergosterol deuteration and Δ 4 cholesten-3-one in cholesterol deuteration. These compounds contained deuterium atoms probably in specific positions, introduced by a reaction similar to the disproportionation reaction.

In these exchange reactions the solvent was heavy water and acetic acid and the extent of deuteration of the methyl group of the acid was investigated over a wide range of exchange reactions. It seemed unlikely that the deuterio acid was an intermediary in sterol deuteration for attempted deuteration of sterols with glacial deuterio acetic acid, containing deuterium atoms in the methyl group, was unsuccessful.

The acetic acid may however have a specific role, for attempted exchange reactions involving sulphuric acid, heavy water and a platinum catalyst gave a sterol product which did not contain deuterium.

Additional Paper on the Combustion of Sulphur to Sulphur Dioxide

This paper contains an account of the combustion of sulphur in the presence of phenol to inhibit formation of sulphur trioxide and to obtain the maximum yield of sulphur dioxide.

A gravimetric method has been developed for estimating sulphites and consequently sulphur dioxide. Using this method of estimation, combustion of sulphur to sulphur dioxide resulted in ninety per cent conversion.

I N D E X

Introduction.

1. The reactions involving exchange between hydrogen and deuterium atoms which have been studied in the last twenty years conform to standard types. The hydrogen atoms taking part in these exchanges may be classified as follows:-

Class I - Ionisable hydrogen atoms exchanging with extreme rapidity.

Class II - Labile hydrogen atoms responsible for tautomeric changes, exchanging rapidly at a measurable rate.

Class III - Incipiently ionised hydrogen atoms exchanging comparatively slowly under the influence, inter alia, of neighbouring electron attracting groups. Within this group appear the exchange reactions involving substituted aromatic compounds.

Class IV - Hydrogen atoms exchanging only with difficulty. Here the hydrogen atoms are regarded normally as stably bound, e.g., paraffin hydrocarbons and aromatic hydrocarbons. Catalytic exchange reactions are invoked to aid exchanges in this class.

The contents of this thesis are concerned largely with class IV reactions and it was from an example of this type of exchange reaction that the research began. The exchange reaction was that between cholesterol and heavy water under the influence of a platinum catalyst. This had been reported by Bloch and Rittenberg in 1943.

The intention in the research was to confirm the preparation of deuterio cholesterol and to extend the method to the deuteration of ergosterol. This completed, the investigation was to be continued in order to gain information on certain factors governing the nature and extent of the exchange reactions between the sterols and heavy water.

The thesis is accordingly divided into the following parts:-

Part I. The preparation of deuterio cholesterol and deuterio ergosterol by a platinum catalysed exchange reaction.

Part II. The deuteration, by a similar exchange reaction, of less complex compounds, each containing a representative structural feature of the sterol molecules.

Part III. The application of the information gained in Part II to the problem of the deuteration of sterols.

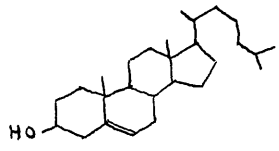
Part IV. The investigation of the role of the solvent employed in these exchange reactions with a note on the role of the catalyst.

Experimental Details for each section of the thesis appear in the corresponding section of Appendix I. Calculations appear similarly numbered in Appendix II.

Part I. The Preparation of Deuterio Cholesterol and Deuterio Ergosterol by a Platinum Catalysed Exchange Reaction.

2. In Part I the intention of the research was to confirm Bloch and Rittenberg's[1] preparation of deuterio cholesterol and to extend the method to the preparation of deuterio ergosterol. Besides extending the method, this would allow some assessment of the influence of their structural differences on extent of deuterium exchange.

In 1937 a paper was published by Rittenberg and Schoenhermer[2] on the attempted deuteration of cholesterol and this was followed by Bloch and Rittenberg's paper in 1943. These authors found that cholesterol



and heavy water would not undergo exchange even at elevated temperatures in the presence of Adams' platinum catalyst.

The addition of acetic acid to the mixture proved efficacious in bringing about exchange. Shaking cholesterol, acetic acid, heavy water and a platinum catalyst for three days at 127°C, produced deuterio cholesterol in 5% - 50% yields. The deuterium was distributed between the ring system of the sterol and the side chain and the amount in the sterol varied between 0.07 and 5.70 atom per cent.

Simultaneously with the exchange, a destruction of cholesterol occurred. The destruction seemed to run parallel to the amount of deuterium introduced into the sterol recovered.

That the function of the acetic acid was more than that of increasing solubility, was shown by the fact that alcohol could not replace the acetic acid in effecting deuteration.

The authors investigated the possibility of exchange in aqueous alcohol acidified with hydrochloric acid but in the absence of platinum. The product did not contain any deuterium.

Using the successful method described, ergosterol and cholesterol were deuterated side by side under similar conditions. This experiment was performed three times. A change in the amount of Adams' catalyst used was the only variation made in each pair of reactions.

It was noted that in the course of the exchange reaction between the sterols and heavy water, the methyl group of the acetic acid present also underwent exchange. The extent of this deuteration was measured in each experiment.

3. General Outline of the Method.

Mixtures of the sterol, platinum (Adams' catalyst), acetic acid and heavy water were sealed in evacuated reaction tubes. These were shaken for three days at 123° to 133°C. The sterols were then extracted, purified and burned in

oxygen: the water formed thus was collected, purified and its deuterium content estimated by the "Gradient Tube" method. The deuterium content of the water was taken as a measure of the deuterium content of the sterols.

The acetic acid solvent was recovered by vacuum distillation and converted to silver acetate. The deuterium content of the acetate was measured, thus indicating the amount of deuterium in the methyl group of the acetic acid.

The data and results are summarised in Tables I, II and III and shown graphically in Fig.1.

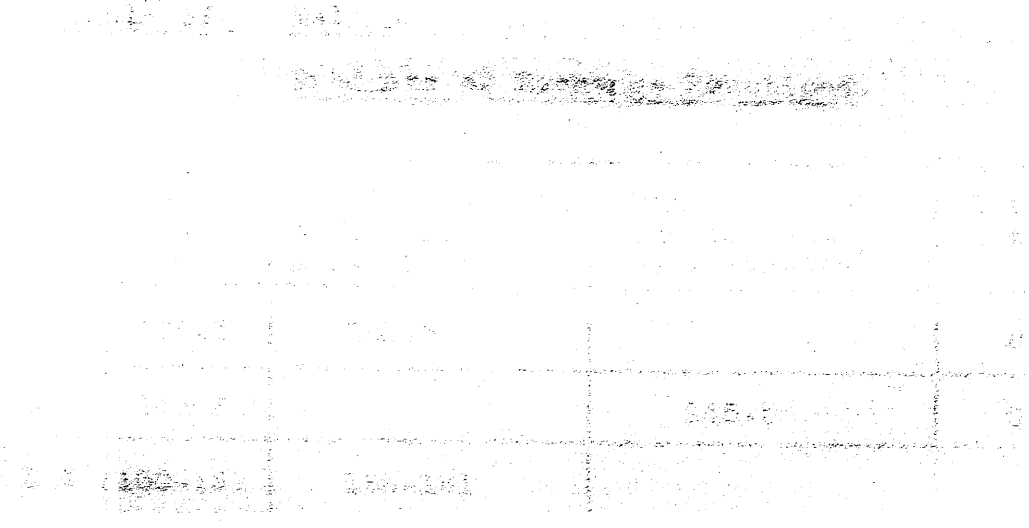


Table I. Deuteration of Ergosterol and Cholesterol.

Contents of Reaction Tubes.

E, 1, 2, 3 Ergosterol. C, 1, 2, 3 Cholesterol.

Expt.		Wt. Sterol	Wt. D_2O	Wt. H_2O	Wt. CH_3COOH	Wt. $PtO_2 \cdot H_2O$
I	E_1	.7975 gm.	.6650 gm.	.1515 gm.	2.8060 gm.	.1270 gm.
	C_1	.7945	.6490	.2090	2.7850	.1220
II	E_2	.7850	.6975	.2075	2.7460	.2320
	C_2	.7925	.6680	.2045	2.7345	.2355
III	E_3	.7870	.6480	.2040	2.7450	.3600
	C_3	.7970	.6520	.2055	2.7180	.3600

Table II. Melting Points (in vacuum) and Weights of Products of Exchange Reactions.

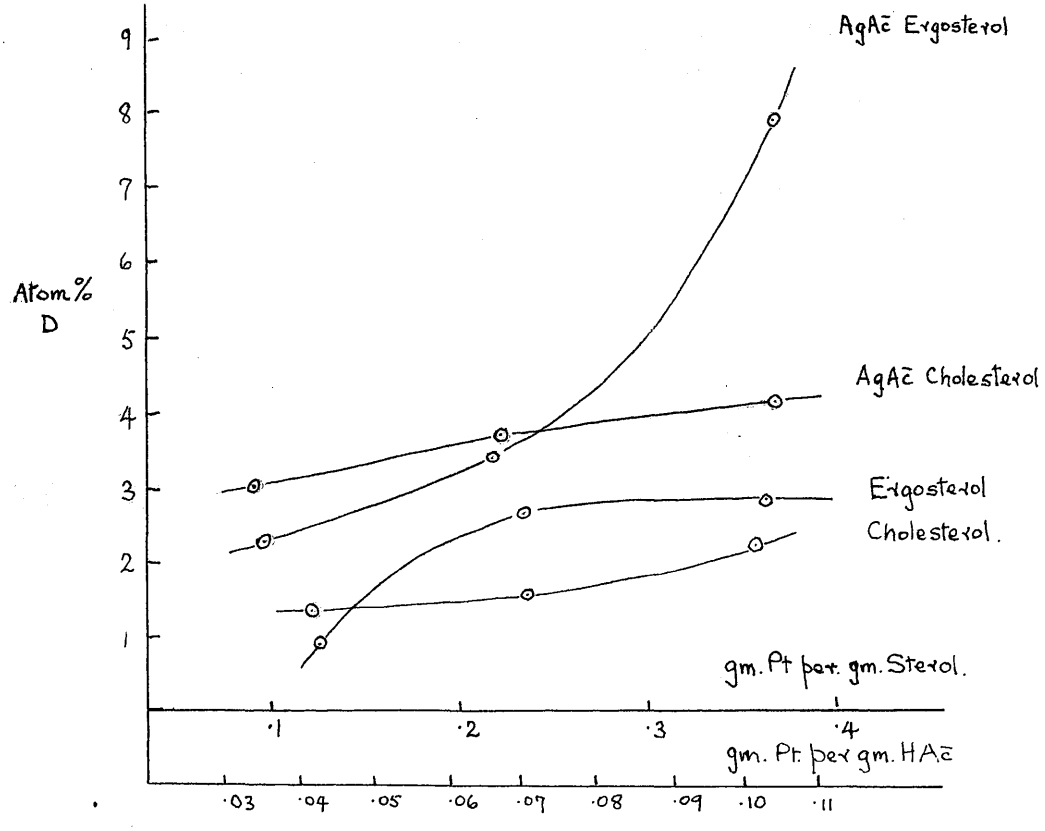
Expt.	M.P. in °C	Mixed M.P. with Ergosterol M.P. 163.5°	Mixed M.P. with Cholesterol M.P. 146-147°	Weight Isolated gm.
E 1	164.3	163.5		0.067
C 1	145.5		145.5	0.090
E 2	150-151	158-161		0.090
C 2	142		144-146	0.140
E 3	160	163		0.029
C 3	145		146-147	0.099

Table III.

Atom % Deuterium in Sterols and Methyl Group of Acetic Acid Solvent.

Expt.	Ergosterol Atom % D	Cholesterol Atom % D	Acetic Acid	
			Ergosterol Deuteration Atom % D	Cholesterol Deuteration Atom % D
1	.93	1.36	2.30	3.06
2	2.66	1.48	3.43	3.65
3	2.79	2.12	7.83	4.12

Fig.1 Graphs Showing the Dependence of Deuteration on Quantity of Catalyst.



Discussion.

These experiments have shown that in the exchange reactions conducted,

(a) deuterio ergosterol may be prepared by means similar to those effective in deuterating cholesterol,

(b) the sterols had suffered considerable destruction,

(c) ergosterol in experiments 2 and 3 had been deuterated to a considerably greater extent than cholesterol. This was probably due to the structural differences between the sterols, i.e., the conjugated carbon carbon double bond system and the single carbon carbon double bond in the side chain of ergosterol compared with the single carbon carbon double bond in ring B of cholesterol.

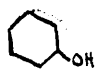
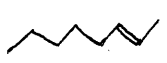
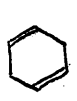
Part II. The Deuteration of Less Complex Compounds.

4. The Influence of Structure on the Extent of the Deuterium Exchange Reaction.

In the deuteration of a complex sterol molecule by a platinum catalysed exchange reaction, it was impossible to predict the positions of deuteration, the mechanism of reaction or even hazard a guess at the parts of the molecule reacting: as this was one of the original objects of this study, and for the sake of finding the information for its own intrinsic value, deuterations were conducted on "parts" of a sterol molecule, each representative substance being chosen to illustrate one feature of the sterol. Saturated and unsaturated aliphatic and alicyclic hydrocarbons, an aromatic hydrocarbon and an alcohol were therefore deuterated under similar conditions in solution.

The substances thus chosen to illustrate the differences in deuteration and relative deuteration due to structural differences are shown in the following table.

Table IV .

Set I.		
	C_6H_{14}	n-hexane
	C_6H_{12}	cyclohexane
	$C_6H_{11}OH$	cyclohexanol
	C_6H_{10}	cyclohexene.
Set II.		
	C_6H_{10}	cyclohexene
	C_5H_{10}	pentene I
	C_8H_{16}	octene I
	C_8H_{16}	octene II.
Set III.		
	C_6H_{10}	cyclohexene
	C_6H_8	1:4 cyclohexadiene
	C_6H_8	1:3 cyclohexadiene
	C_6H_6	benzene.

5. The hydrocarbons were deuterated in three sets of four experiments each and cyclohexene appeared in each set to provide a common reference. Having completed these three sets of deuteration experiments, the whole procedure was repeated under similar conditions, the only difference being in the ageing of the catalyst. Two complete groups of results are therefore available, Group I and Group II.

The quantity of hydrocarbon used in each experiment was approximately 1/28th of a gram molecule and the quantities of the other constituents of the reaction tubes were substantially the same in all cases.

The hydrocarbons, together with the platinum black catalyst, heavy water and acetic acid were weighed into "Pyrex" brand tubes. The contents were frozen by immersing the tubes in liquid air or in a solid carbon dioxide - acetone freezing mixture, the tubes evacuated with a "Hyvac" pump, sealed and shaken in a heated shaker for three days at 123°C - 133°C. After this treatment the hydrocarbons were isolated and their deuterium contents estimated. The deuterium entering the methyl group of the acetic acid was also determined in each case.

The deuterations were carried out in acetic acid solution so that the conditions might be the same as those described for the preparation of deuterio sterols.

In some cases a few drops of polymer were recovered

after the exchange reactions. These were isolated and their deuterium contents measured.

6. Results.

Since equimolecular quantities of the hydrocarbons were used, probably the best method of comparing the results is to express them as the total number of deuterium atoms transferred into the hydrocarbons.

This method measures the effect of the characteristics of each molecule on deuterium exchange. Atom % measurements could on the other hand lead to equating the deuterium exchange of compounds such as

$CH_3(CH_2)_nCOOD$ and $C_6H_{11}D$.

In the case of the carboxylic acid although facile carboxyl hydrogen has taken place and a large number of deuterium atoms could be introduced into this position, the atom % value might well equal that of a cyclohexane sample which exchanges its hydrogen atoms only with difficulty.

Table V.

Reaction Mixtures (Weights in gm., volumes in ml.) and Results (as % D_2O weight/weight).

Hydrocarbon	Catalyst Wt.	D_2O Wt.	$HA\bar{c}$ Wt.	Hydrocarbon Wt.	Hydrocarbon Vol.	% by Wt. D_2O Hydrocarbon	% by Wt. D_2O $Ag\bar{A}c$	Expt. No.
	.0805	.427	1.635	3.1055	4.6	0.41	13.71	1
	.0810	.427	1.636	3.0105	3.8	.31	10.78	2
	.0810	.433	1.653	2.9340	3.87	1.69	2.84	3
	.0780	.400	1.532	2.7755	3.4	9.53	1.73	4
	.0810	.4315	1.648	2.9540	3.6	9.18	5.33	5
	.0810	.429	1.641	2.5665	3.85	2.96	12.63	6
	.0810	.4325	1.651	3.9765	5.57	2.21	10.64	7
	.0810	.4305	1.644	3.9760	5.52	1.96	9.41	8
	.0810	.4350	1.645	2.9725	3.67	8.37	2.75	9
	.0810	.4335	1.640	2.8405	3.32	10.41	2.23	10
	.0810	.4367	1.651	2.8545	3.38	2.45	4.63	11
	.0810	.4335	1.640	2.7705	3.19	14.07	1.78	12
	.0810	.4311	1.646	3.1280	4.6	.29	11.18	21
	.0810	.4317	1.648	3.0335	3.8	.07	10.35	22
	.0810	.4385	1.674	3.0170	3.87	.64	2.66	24
	.0810	.4328	1.653	2.8080	3.42	.77	6.70	23
	.0810	.4317	1.650	3.0010	3.6	.66	7.59	25
	.0810	.4328	1.654	2.5630	3.85	1.39	7.84	26
	.0810	.4321	1.651	3.9770	5.57	1.73	7.32	27
	.0810	.4313	1.648	3.9730	5.52	1.37	8.94	28

Table V (Contd.)

Hydro-carbon	Catalyst	D_2O	$HA\bar{c}$	Hydrocarbon		% by Wt. D_2O		Expt. No.
				Wt.	Vol.	Hydro-	$AgA\bar{c}$	
	.0810	.4340	1.653	2.9885	3.6	8.67	5.33	17
	.0810	.4308	1.643	2.8525	3.32	9.16	4.66	18
	.0810	.4319	1.646	2.8325	3.38	1.85	8.29	19
	.0810	.4321	1.647	2.7900	3.19	13.97	3.02	20

Table VI

Boiling Points of Hydrocarbons in Degrees Centigrade.

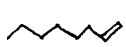
Expt.	Hydrocarbon	B.P. (°C) before deuteration	B.P. (°C) after deuteration	Colour of Hydrocarbon: Starting Material	Colour of Hydrocarbon: After Deuteration	Colour of Hydrocarbon: After Purifying	Polymers formed
1		68	67-68	colourless — All clear	Nil	All colourless	Nil
21		68	67-68		Nil		Nil
2		80-80.2	80		Nil		Nil
22		80-80.2	80-80.5		Nil		Nil
3		161	158-160		Nil		Nil
24		161	160-163		Nil		Yellow
4		83-83.5	78		Nil		Nil
23		83-84.5	81-84		Yellow		Amber
5		83-83.5	78		Nil		Nil
25		83-84.5	81-84		Yellow		Amber
6		38-39	38-39		Nil		Nil
26		38-39	37-39		Wine red		Amber
7		119-121	120-122.5		Pale yellow		Oily yellow
27		120-121	120-122		Pale yellow		Yellow
8		122-124.5	123-124.5		Clear		Nil
28		123.5-125	122-124		Pale yellow		Yellow
9		83-84.5	77.5-78.5		Nil		Nil
17		83-84.5	77-79		Nil		Nil
10		87	77-78		Nil		Nil
8		87	77-78		Nil		Nil
11		83-86	80-84		Yellow		Brown
19		83-86	80-84		Brown		Brown
12		80-80.5	78		Nil		Nil
20		80-80.5	77		Nil		Nil

Table VII.

Table of Comparison of Deuteration of Hydrocarbons.

Expt. No		Compound	Total D atoms transferred into hydrocarbon (x 100 N)		Total D atoms transferred into acetate (x 100 N)	
Set I	Set II		Group I	Group II	Group I	Group II
2,	22		.121	.027	.802	.775
1,	21		.18%	.133	1.02	.836
	23		isomer-	.238		.502
	25		ised	.218		.568
3,	24		.534	.209	.224	.203
11,	19		.635	.473	.346	.618
6,	26		.980	.458	.945	.588
8,	28		1.01	.717	.702	.668
7,	27		1.13	.885	.798	.548
10,	18		2.70	2.37	.165	.347
12,	20		2.74	2.73	.132	.224
4,	17		2.93	2.87	.120	.399
5			3.00		.398	
9			2.76		.204	

Table VIII.

Table of Comparison of Deuteration of Hydrocarbons.

R	Total D atoms into R / Total D atoms into Ac'		Total D atoms into R / Total D atoms into R + Ac'	
	Group I	Group II	Group I	Group II
	.151	.035	.131	.034
	.185	.159	.156	.137
	isomer-ised	.476 .384		.321 .280
	2.38	1.04	.704	.507
	1.84	.766	.647	.433
	1.04	.780	.510	.437
	1.44	1.07	.590	.517
	1.42	1.62	.586	.617
	16.42	6.83	.942	.872
	20.8	12.2	.955	.975
	24.6 7.55 13.53	7.20	.965 .885 .932	.880

Table IX.

% by Wt. D_2O Found in Combustion Water from Polymers.

Expt. No.	% D_2O by wt.	Colour
11 	3.79	brown
19 	6.76	brown
23 	3.22	amber
26 	6.39	amber

7. Calculation to Investigate Whether or Not the Total Number of Deuterium Atoms Introduced into the Hydrocarbons and Acetate Radicles was a Constant.

A study of the total deuterium transferred was made, for it was noted that a high deuterium content in the hydrocarbons was accompanied by a low content in the acetate radicle and vice versa. A calculation was made of the deuterium atoms transferred in several cases.

Results Table X.

Hydrocarbon	Expt.	Deuterium Content		Total
		Hydrocarbon	Ac'	
	1	.00187 N	.01022 N	.01209 N
	2	.00120 N	.00802 N	.00922 N
	23	.00238 N	.00502 N	.00740 N
	6	.00980 N	.00944 N	.01924 N
	7	.01133 N	.00799 N	.01932 N
	8	.01005 N	.00701 N	.01706 N
	20	.02743 N	.00225 N	.02968 N
Isomerised 	9	.0276 N	.00204 N	.02964 N

From these results it can be seen that:-

a. The total number of deuterium atoms transferred into the hydrocarbon and acetate radicle is not a constant.

b. There is a general increase in the total deuterium transferred into hydrocarbon and acetate in the series cyclene, cyclane, alkane, alkene, benzene.

8. Alkane and Cyclane Deuteration.

In the saturated cyclohexane and n-hexane molecules the carbon bound hydrogen atoms, uninfluenced by neighbouring hydroxyl groups or carbon carbon double bonds, had exchanged to a relatively small extent.

The possibility of dehydrogenation by the catalyst has been examined by the measurement of bromine absorption values. No dehydrogenation was detected.

Symmetry of the molecule probably accounted for the lower deuterium content of the cyclane.

9. Cyclohexanol Deuteration.

The method used to isolate the cyclohexanol may have resulted in some exchange of the deuterium atoms of the product.

In general in all the results recorded a high percentage deuterium in the acetate radicle has been accompanied by a low content in the hydrocarbon and vice versa.

An acetate content of 2.45 atom % in this experiment suggested a higher value for the cyclohexanol.

10. Alkene and Cyclene Deuteration.

The introduction of one carbon carbon double bond into

the molecules has resulted in greater deuterium exchange. The introduction of two C:C bonds into the molecule in the case of 1:3 cyclohexadiene resulted in slightly more than twice the number of deuterium atoms being transferred into the molecule than in the case of the single C:C bond compounds, c.f., cyclohexene expts. 23 & 25.

That this is so is probably due to the conjugation of the C:C bonds in the di-ene. This is substantiated in the benzene results where the introduction of three conjugated C:C bonds into one molecule has resulted in such facile hydrogen atom replacement that a state approaching equilibrium had been attained between the benzene and heavy water hydrogen atoms.

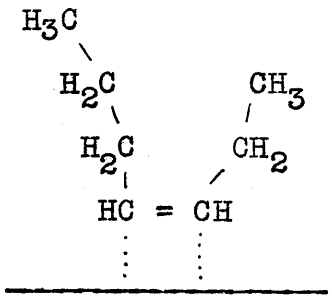
11. <u>The Prima Facie Importance of the C = C in deuterations by a platinum catalysed exchange reaction.</u>

The presence of such a group as -CN or -COOH in a molecule together with a C = C group leads to deuterium exchange in alkaline solutions. The unsaturated nitrile $CH_2 - CH = C - CH_2 - CN$ exchanges rapidly in heavy alcohol - $CH_2 - CH_2 - CH_2$ sodium ethoxide solution (5) and the unsaturated acid, $CH_2 = CH\ COOH$, undergoes exchange in alkaline heavy water. Many more examples of this type of exchange might be quoted.

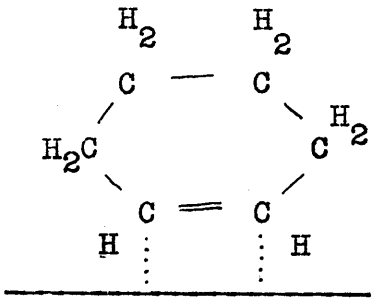
In those molecules which contain the C = C bond alone exchange normally requires the presence of a catalyst. After

twenty seven hours, equilibrium was reported for ethylene and heavy water at 80°C with a nickel catalyst (4). A mechanism suggested by Polanyi depended on chemisorption of the C = C group on the catalyst with bond opening, transfer of a hydrogen atom and either removal or addition of a further hydrogen atom (4).

If C = C adsorption is of prime importance, then in the deuteration of two ethylenic hydrocarbons of different chain length, provided all other conditions are the same, there should be an equivalence between the amount of deuterium introduced into each hydrocarbon



Catalyst



Catalyst

The deuteration in these two cases should not be dependent on the chain length or configuration.

In the series of experiments conducted under section 4 several olefinic hydrocarbons were included.

Cyclohexene, Pentene I, Octene I, Octene II,

As already stated, 1/28th of a gram molecule of each hydrocarbon was shaken with heavy water, a "platinum black"

catalyst and acetic acid in a sealed, evacuated tube for three days at 123° - 133°C. The hydrocarbons and the acetic acid solvent were then isolated and their deuterium contents estimated.

The results shown in the following table indicated that the chain length and orientation had considerable effect in determining the extent of deuteration, though this was in general greater than that found in the deuterio saturated hydrocarbons.

Table XI.

The total number of deuterium atoms transferred into the alkenes and the cyclene.

Hydrocarbon	No.D atoms transferred into hydrocarbon R (mean for two expts. x 100N)	No.D atoms transferred into R / No.D atoms transferred into R & Ac'
	.228	.300
	.721	.474
	.863	.554
	1.008	.602

Had the deuteration of an olefine depended solely on the C:C bond then the numbers of deuterium atoms transferred would have been the same in each case, although the results expressed as atom % would have differed.

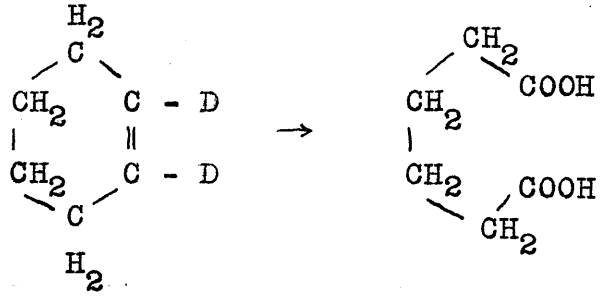
The number of deuterium atoms transferred increased in the series cyclene, C5 alkene, C8, 2:3 alkene, C8, 1:2 alkene. The cyclic olefine was least susceptible to exchange and as chain length increased so also did the ease of deuteration. Change in position of the C:C bond from the 1:2 position to the 2:3 position resulted in slightly decreased exchange.

Calculations showed that the deuterium content of the alkenes corresponded to values for less than even one hydrogen position in equilibrium with the solvent.

12. The Location of the Deuterium Atoms in Deuterio cyclohexene Prepared by a Platinum Catalysed Exchange Reaction.

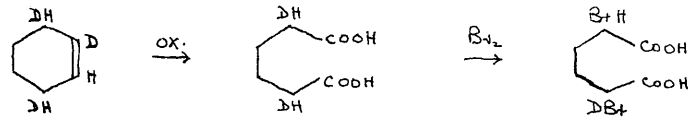
In the hope that the distribution of deuterium atoms would cast some light on the mechanism of the exchange reaction, experiments were conducted on the deuterium location in deuterio cyclohexene.

The method employed was to convert deuterio cyclohexene to adipic acid and crystallise the acid from a large volume of water



By this means deuterium atoms in the positions shown would be removed and a deuterium assay before and after the conversion would indicate the number of deuterium atoms in these positions.

A sample of deuterio adipic acid was then converted to $\alpha\,\alpha'$ dibromadipic acid and a comparison made of the deuterium content of the two acids. Assuming random replacement of hydrogen or deuterium atoms by bromine atoms, information was obtained about the deuterium atoms in positions "α" and "β" to the double bond in deuterio cyclohexene.



The preparation of suitable samples of deuterio cyclohexene is described in the Appendix. The conversions to adipic acid were accomplished by two different methods and the results compared, lest the conversion methods should have caused exchange of other deuterium atoms present.

The first method employed potassium permanganate and silver nitrate in aqueous solution. The concentration of hydroxyl ions was thereby limited by silver oxide or hydroxide precipitation. The adipic acid product was acidified and extracted with ether and crystallised from water. The objection to the use of permanganate oxidation is that this treatment is reputed to isomerise carbon-carbon double bonds and structural conclusions are not made from this degradation unless the double bond is resistant to platinum catalysed hydrogenation in acid solution.

The second conversion method to be used avoided this objection by converting the cyclohexene to cyclohexanediol with hydrogen peroxide and osmium tetroxide.[43] The diol was then converted to adipic acid by permanganate oxidation.

Bromination of adipic acid to produce $\alpha\alpha'$ dibromoadipic acid was conducted in thionyl chloride solution.[44]

Table XII.

Expt. No.	Conversion by	Atom % D in		
		Cyclohexene	Adipic Acid	Dibromo Adipic Acid
1L	$KMnO_4$ H_2O_2	.83	.60	--
2L	H_2O_2 OsO_4	.94	.59	--
3L	do.	.69	.59	.56
11A	do.	1.42	1.24	1.04
12A	do.	1.67	1.51	1.37

Table XIII.

Deuterium Distribution. Figures for One Molecule Cyclohexene.

Expt.	Cyclohexene	4α and 4β positions	CH:CH positions	4 α positions	4β positions
1L	.083	.060	.023	--	--
2L	.094	.059	.035	--	--
3L	.069	.059	.010	.029	.030
11A	.142	.124	.018	.082	.042
12A	.167	.151	.016	.083	.068

Expts. 11&12A were conducted by Mr.A. Macdonald.

The results of the investigation, together with other experimental results described in later sections, suggest that in platinum catalysed deuterations the double bonds of the molecules may be mobile or that the "Hydrogen Switch" mechanism is an acceptable description of the exchanges. This mechanism is discussed more fully in Section 31.

13. Deuteration of Benzene.

The possibility of an equilibrium exchange state being reached between the hydrogen atoms of the benzene and the deuterium atoms of the heavy water has been investigated by calculation.

It was assumed that there was an even distribution of the deuterium atoms of the heavy water between the following hydrogen positions:-

a. in the water molecules,

b. in the carboxyl groups of the acetic acid,

c. in the benzene molecules.

The deuterium atoms in the methyl group were considered to be removed from the equilibrium system.

The calculated result for the atom % deuterium to be expected in the benzene was 14.35%. The value found in experiment 20 was 12.75%, a value only slightly less than the equilibrium value.

14. The Disproportionation Reaction and Deuteration.

In conducting the experiments on the deuteration of the hydrocarbons shown in Table IV it had been observed that there were changes in the boiling points of several hydrocarbons after the exchange reaction. The boiling point of the product in cyclohexene deuterations was in several experiments, though not in all, several degrees below that of the starting material. The same observations might be made about the product in the deuteration of 1:4 cyclohexadiene.

Accompanying these changes were unexpectedly high deuterium contents of the products.

Should disproportionation or isomerisation have occurred during the exchange reaction, the results obtained would not represent extent of exchange alone. They would represent the result of exchange followed by isomerisation or vice versa. n-Hexane, cyclohexane, pentene and octene have all been isomerised by catalytic and non catalytic treatments (6). The temperatures noted for these isomerisations however were in the region 300°C to 500°C and it was assumed that the conditions of the reactions discussed in this report would be unlikely to produce isomerisation.

The hydrocarbons used which were more likely to isomerise during deuterations were the cyclo mono- and di-olefines. The benzene used was unlikely to isomerise for it appears as

an end product in cyclic hydrocarbon isomerisations.

The possibility of disproportionations or isomerisations during these deuteration reactions was therefore considered.

That disproportionation occurred has been established by examining the double bond content of the hydrocarbons by bromine absorption and by chemical separation of the products. Calculation methods have also been applied to the problem.

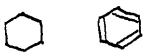
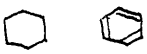
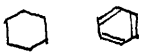
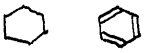
This theory accounts for the anomalous results found in the deuteration of cyclohexene in experiments 4, 5, 9 and 17 and 1:4 cyclohexadiene experiments 10 and 15.

In these cases a comparatively high deuterium content was found in the products together with a fall in boiling point.

15. Bromine Absorption Values of Hydrocarbons Before and After the Exchange Reactions.

Any isomerisation of the hydrocarbons in the deuteration mixtures would have resulted in a change of bromine absorption values of the hydrocarbons after deuteration. Table XIV shows the suggested isomers formed and the observed bromine absorption values.

Table XIV.

Expt.	Hydrocarbon	Suggested products	Atom % D in product	Double bonds per molecule	
				predicted	found
23			.695	1	.91
25			.595	1	.93
17			7.78	0	.18
9			7.58	0	.01
11			2.22	1	.56
19			1.67	1	.59
18			8.32	0	.10
10			9.52	0	.04
27			1.56	1	.97
22			.06	0	.00

16. Notes on Bromine Absorption Values.

a. Cyclohexene and 1:4 cyclohexadiene. The absorption values and the double bond contents were as predicted. Cyclohexene disproportion had occurred in some cases and not in others.

b. 1:3 cyclohexadiene. The unexpected result of about .5 D.B. might be explained as follows. Zelinsky and Pavlow[7] found that the diene could be changed by platinum even at room temperature. After the reaction had proceeded for a few minutes no diene could be detected and the bromine absorption value had fallen by 50%. (1:3 cyclohexadiene rapidly adds 1 molecule of Br_2 at room temperature.)

It was concluded that the reaction took place by two stages.

$$2\ C_6H_8 \rightarrow C_6H_6 + C_6H_{10} \qquad \text{(rapid)}$$

$$3\ C_6H_{10} \rightarrow C_6H_6 + 2\ C_6H_{12} \qquad \text{(slow)}$$

The deuteration results for the 1:3 diene may therefore be the result for the deuteration of a mixture of cyclohexene, benzene and cyclohexane together with any remaining diene.

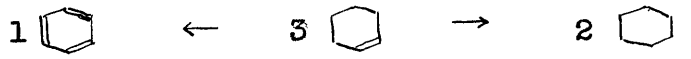
The comparatively low deuterium content of the 1:3 diene suggests however that no isomerisation occurred for the deuterium content was hardly more than twice the value for an unisomerised sample of cyclohexene. Had disproportionation occurred a much higher value would have been expected.

c. Cyclohexane. As far as could be observed no dehydrogenation of the starting material had occurred although the deuteration might proceed via a dehydrogenated stage of the cyclohexane.

d. Octene I. Double Bond character of the product was investigated to examine the possibility of hydrogenation.

17. Disproportionation of Cyclohexene.

It was found by Zelinsky and Pavlow about 1924 (8) that cyclohexene passed over a palladium catalyst at 92°C in an atmosphere of carbon dioxide produced the following reaction:-



Also cyclohexene passed over a palladium catalyst at 164°C and 223°C gave a product with R.I. n_D^{20} 1.4448 and n_D^{20} 1.4489 respectively.

They stated that pure cyclohexene has R.I. n_D^{20} 1.4460 and instead of this rising to the value n_D^{20} 1.5010 for benzene the value decreased. This led to an investigation of a mixture of benzene and cyclohexane in the ratio 1 : 2. This had R.I. n_D^{20} 1.4455.

R.I. measurements were made on cyclohexene expt.5, before and after the exchange reaction. The values obtained were n_D^{18} 1.44835 and n_D^{18} 1.44218, indicating that a change had occurred during the deuteration.

Two possible mechanisms were examined to account for the deuterium content of the product.

Mechanism I. The equation $3C_6H_{10} = 1\ C_6H_6 + 2\ C_6H_{12}$ represents the disproportionation. In this mechanism it was assumed that disproportionation took place without deuterium exchange during the early stage of the reaction.

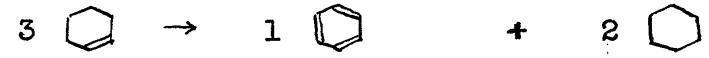
A calculation showed that if it was assumed that:-

(a) the benzene was in equilibrium with the heavy water, at the end of the reaction,

(b) the cyclohexane was deuterated hardly at all in conformity with experiments on the deuteration of cyclohexane, then the atom % deuterium in the product should be 5.49 atom %, whereas the experimental value was 8.34 atom %.

This unsatisfactory result led to the examination of a second mechanism.

Mechanism II. In this explanation it was assumed that in the disproportionation,



(a) four atoms hydrogen were removed from the cyclohexene to produce benzene,

(b) two hydrogen or deuterium atoms from the solvent were added to each cyclohexene molecule to produce cyclohexane,

(c) the deuterium content of the benzene reached the equilibrium value with the heavy water left after the disproportionation,

then:-

Atom % D expected in product = 7.53%

Atom % D found in product = 8.34%.

This mechanism seemed to be a fair representation of the disproportionation and deuteration.

18. Cyclohexene Disproportionation.

The deuteration of various samples of cyclohexene.

In previous experiments on deuteration of cyclohexene by a platinum catalyst exchange reaction in some cases only had a disproportionation occurred. This conclusion was drawn because of the lowering of the boiling points of the products of deuteration and because of the change in the bromine absorption values of starting material and product.

As a preliminary step in investigating the anomaly of disproportionation in some cases and not in others, deuterations were performed on several samples of cyclohexene. In this set was included a reaction tube containing ordinary water and cyclohexene to investigate the possibility of disproportionation in the absence of heavy water. The results showed that disproportionation did occur in this case.

It has been found that disproportionation occurred in deuterating freshly distilled cyclohexene and did not occur when the sample had aged and been stabilised by a change in the cyclohexene. Freshly distilled samples showed the theoretical double bond content and ageing resulted in a slight fall in the bromine absorption value, perhaps the result of some atmospheric oxidation.

The reactions were conducted in a similar manner and with similar quantities to those prepared previously.

Table XV.

Bromine Values and Boiling Points. Days between distillation and deuteration.

Expt. No.	Boiling Points		Bromine Values		"Age" in Days
	Starting Material	Product	Starting Material	Product	
29	83-84.5	82.8-84	.96	.94	200
30	82.5-82.8	83 - 84	1.02	.01	1
31	82.8-83.5	80	1.01	.01	1
32	82.5-82.8	77.5-78	1.02	.02	1

Table XVI

Deuterium Contents.

Product Expt. No.	Atom % D	Atom % in CH_3 of solvent
29	.94	7.74
30	9.18	1.37
31	9.20	1.60
32	.04	.03

19. Disproportionation of 1:4 Cyclohexadiene.

The Deuterium Content of the Separated Products Formed by the Disproportionation of 1:4 Cyclohexadiene.

In the deuteration of cyclohexadiene it was suspected that a mixture of benzene and cyclohexane had been produced:-

$$3\ C_6H_8 = 2\ C_6H_6 + C_6H_{12}$$

The products of the deuteration were separated into two portions by treatment with 30% oleum. The paraffin hydrocarbon floated unchanged on top and the benzene was recovered as sodium benzene sulphonate.

The atom percent deuterium of the samples was:

(a) initial mixture benzene and cyclohexane	8.32%
(b) separated benzene fraction	3.82%
(c) separated cyclohexane fraction	11.70%
(d) methyl group of the acetic acid solvent	4.22%.

The results were quite unexpected for straightforward deuteration of cyclohexane had produced a result of 0.28%. Deuteration of benzene had produced a result of 12.85%.

Calculations showed that:-

(i) if the overall percentage of deuterium in the product was 8.32 atom % and that of the cyclohexane was 11.70 atom %, then the calculated quantity in the benzene was 4.97%. The experimental result was 3.82 atom %,

(ii) the benzene hydrogen atoms did not reach the equlibrium value in the exchange of 10.66 atom %,

C.J.L.28

(iii) the hydrogen or deuterium atoms added to the cyclohexadiene came from the solvent. The calculated atom % deuterium in the cyclohexane was 13.92 atom %. The value found by experiment was 11.70%.

The conclusion drawn about the mechanism of reaction was that the atoms added to the cyclohexadiene molecules were selected at random from the solvent. Secondly the removal of H atoms from cyclohexadiene to produce benzene seems to preclude the catalyst bringing the exchange value for the benzene to the equilibrium value.

20. Total Deuterium Transfer and Disproportionation.

Further evidence in favour of the disproportionation of certain hydrocarbons was to be found in a consideration of the total deuterium transfer into acetate radicle and hydrocarbon in the previous experiments.

Table XVII.

Hydrocarbon	Values x 100 N
Cyclene	.763 (mean value)
Cyclane	.863
1:3 cyclohexadiene	1.036
Alkene	1.572
Benzene	2.931
"Cyclohexene"	3.168
"1:4 cyclohexadiene"	2.791

The values for the last three classes of compound are so close as to suggest deuteration of either closely similar or identical compounds, when the results are compared with the lower values for the previous four types.

21. General Conclusions Part II.

As a result of the experimental work described in this Part, the following conclusions were drawn:-

(1) The saturated parts of the deuterio sterol molecules were unlikely to contain more than a small fraction of the deuterium atoms in the molecule.

(2) The deuterium atoms were likely to be distributed

(a) on the carbon atoms of double bonds or in positions " α and β " to these bonds,

(b) near the alcoholic group of the sterols.

(3) Redistribution among different molecules of hydrogen atoms by the catalyst in disproportion reactions resulted in considerable deuteration of the products together with destruction of the starting material.

22. Part III. The Deuteration and Isomerisations of the Sterols.

The foregoing results suggested that it would be profitable to re-examine the deuteration of the sterols since it had been noted that an increase in the number of double bonds in the sterol molecule increased deuterium exchange.

The exchange mechanism may therefore be connected with possible hydrogen atom redistribution under the unfluence of the catalyst. This process would be analogous to Disproportionation in which the catalyst removed hydrogen atoms from one molecule and donated them to others. For the sterols an analogous reaction would be the removal of hydrogen atoms from one ring of the sterol and their donation to another, a process involving the migration of carbon carbon double bonds and the production of isomers of the original sterol.

When the sterols ergosterol and cholesterol were then deuterated using an active platinum on asbestos catalyst, definite evidence of the migration of double bonds and the production of isomers was obtained.

If deuteration and bond migration have a common mechanism then the increased destruction of sterol would naturally accompany a high deuterium content in the recovered unisomerised sterol. This latter phenomenon was observed by Bloch and Rittenberg. The destruction of sterol which they noted

and that which is recorded in the early parts of this thesis may therefore be accounted for by isomerisation.

The deuterium content of the isomers produced would be accounted for by the addition of deuterium atoms from the solvent, during the isomerisation process, just as deuterium atoms were introduced into the cyclohexane during cyclohexadiene disproportionation in heavy water.

23. Ergosterol Deuteration and Isomerisation.

The recovery of deuterio ergosterol from preparations by an exchange reaction under the influence of Adams' catalyst had been poor (Part I). This section contains an account of an experiment conducted to ascertain the nature of any transformation products formed during the exchange reactions. The catalyst used was platinum black on asbestos.

Ergosterol, acetic acid, heavy water and ordinary water were shaken with a platinum catalyst in an evacuated sealed tube for three days at 124-133°C. After opening the reaction tube, the water and the acetic acid, together with any volatile material produced, were removed by vacuum distillation. The weight of the liquids recovered was less than that of the liquids weighed into the tube before deuteration. It was assumed that this was due to the formation of ergosteryl acetate.

The solid products of the deuteration were then separated from the catalyst by dissolving them in chloroform and filtering off the catalyst. The recovery of the catalyst was quantitative and the recovery of the solids slightly greater than expected. This was probably due to the formation of a gum by the solids and the chloroform.

The solid products were dissolved in benzene and chromatographed on alumina in an attempt to separate the components. Some 30 fractions were isolated by this method and recovery of

solids was quantitative. The products of the chromatograph were gums and oils.

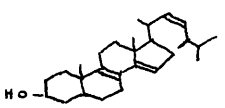
Repeated attempts at crystallisation produced crystals from one fraction only. Re-chromatographing one of the samples produced more gums, of which one only yielded a few crystals.

Examination of Ultraviolet Absorption Spectra.

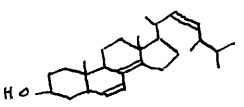
Towards an investigation of the nature of the products from the first chromatographic analysis, three of the fractions were taken and an examination made of their absorption of ultra-violet light. The fractions taken were from the initial, middle and final fractions of the chromatograph.

The absorption maxima, fig.4, lie in the region 230-250 mμ and this suggested that the sterols produced contained heteroannular conjugated double bonds. (C:C - C:C distributed between two rings). Homoannular di-enes absorb in the region 265-285 mμ (9).

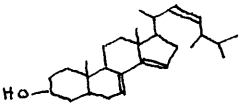
A search of the literature for other isomerisations of ergosterol elicited the information that the isomerisation could be achieved by the action of dry hydrochloric acid gas on ergosteryl acetate. The products were the sterols



Ergosterol B 1
m.p. 148°
$[\alpha]_D$ -40°
λ_{max}^{ether} 248 mμ.



Ergosterol B 2
m.p. 126°
$[\alpha]_D$ -88°
λ_{max}^{ether} 248 mμ.



Ergosterol B 3
m.p. 136°
$[\alpha]_D$ -207°
λ_{max}^{ether} 242 mμ.

The absorption maxima suggested that a similar isomerisation may have occurred during the deuteration of ergosterol. No absorption maxima appear in the region 260-280 mμ that value characteristic of a sterol containing an aromatic ring system. The possibility of the production of an aromatic system by disproportion was therefore eliminated.

An examination of the products of deuteration was made to isolate any sterols which might be characterised. Fraction number 10 from the chromatograph was characterised by the data shown below. This was the only fraction from which crystals were obtained.

Characteristics of Product and Ergosteryl B 1 Acetate.

Product, Fraction 10.	Ergosteryl B1 Acetate. (Windaus)
m.p. crude substance 131°C	132-135°C
Final m.p. 135-136.5°C	142°C
$[\alpha]_D^{18}$ -50.9°	$[\alpha]_D^{19}$ -53.6°
$\lambda_{max}^{CHCl_3}$ 248 mμ for acetate, fig.5.	λ_{max}^{ether} 248 mμ for alcohol
crystals in elongated narrow plates.	needles

C.H. Analysis

found: C, 81.86% H, 10.34%

calc.: C, 82.13% H, 10.57%.

These results suggested that one of the products of the deuteration of ergosterol was ergosteryl B1 acetate.

The remaining fractions of the chromatograph were treated according to the method of Windaus (10), to attempt a separa-

tion of any other of the isomers present. No isomer corresponding to B2 was isolated but an adduct was isolated with maleic anhydride in a small quantity, possibly the adduct of ergosterol B3.

This isomerisation of ergosterol to ergosterol B1 would allow a direct introduction of deuterium in the sterol, probably in the 5, 6 and 7 positions.

This is one of several comparable catalytic bond migrations observed with other sterols.

Δ 7 cholestenol can rearrange to the Δ 8:14 cholestenol (33). Wieland and Benend (34) have pointed out that migration occurs with platinum catalysts in an acidic medium, acetic acid, but not in ethyl acetate, a neutral medium. Stavely and Bollenback (35) however, and Barton (4) state that the migrations of Δ 7 to Δ 8,14 double bonds only occurs when the catalyst is saturated with hydrogen, a condition not necessarily obtaining in the experiment just described.

24. Ergosterol Deuteration and Isomerisation.

In section 23 a description was recorded of the isolation of ergosteryl B1 acetate during an attempt to introduce deuterium into ergosterol by a platinum catalysed exchange reaction. No ergosterol was recovered from the preparation and the quality of the B1 acetate isolated, left some room for improvement. In the experiment which is the subject of this section, the yield was increased and the quality of the B1 acetate improved.

The procedure adopted in this experiment was slightly different from that used previously. The mixture of ergosterol, water and heavy water, acetic acid and platinum black on asbestos were shaken together at about 127°C for three days. The solvents were then removed by distillation and the solids recovered by dissolving them in hot alcohol and filtering off the catalyst. The use of chloroform for this purpose was avoided as its use seemed to encourage the formation of gums difficult to crystallise.

Repeated crystallisation of this alcoholic extract produced a 10% yield of ergosterol B1 acetate.

Product	Windaus et al. (10) Ergosteryl B1 acetate
m.p. 139.5-141°C	142°C
$[\alpha]_D^{18.5}$ -56.1°	$[\alpha]_D^{19}$ -53.6°
λ_{max}^{ether} 249 mμ	λ_{max}^{ether} 248 mμ for ergosterol B1.

Deuterium Content.

Atom % deuterium in ergosterol B1 = 3.68%

Atom % in methyl group of acetic acid solvent= 11.08%.

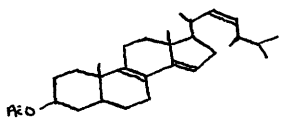
Carbon Hydrogen Analysis.

Found for product	C, 82.32% ;	H, 11.17%.
Calc. for ergosteryl B1 acetate	C, 82.13% ;	H, 10.57%.

25. Isomerisation of Ergosteryl B1 Acetate.

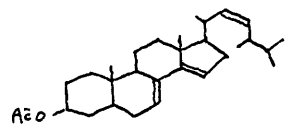
In addition to isolating the ergosteryl B1 acetate an attempt was made to isomerise it to ergosteryl B3 acetate. The procedure adopted was that suggested by Windaus (10).

A portion of the ergosteryl B1 acetate was treated with dry hydrochloric acid gas in dry chloroform. Addition and removal of hydrochloric acid was expected to isomerise one of the C:C double bonds as follows:-



HCl

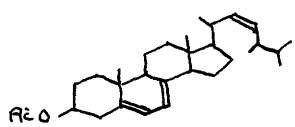
1,2 addition
1,2 elimination

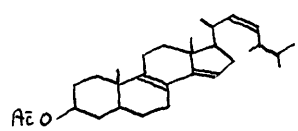


Ergosterol B1 Acetate

Ergosterol B3 Acetate

By this means it was expected that any deuterium atoms present in the "7" position in the B1 acetate would be eliminated and that the deuterium content of the sterol would fall. To suggest that there should be deuterium in the "7" position is not unreasonable for the transformation





Ergosteryl Acetate

Ergosteryl B1 Acetate

probably results in addition of deuterium or hydrogen atoms from the solvent into positions 5, 6 and 7.

A very small sample of the ergosteryl B3 acetate was obtained with the following characteristics:-

	Windaus et al.
m.p. 129.5-130.5°C	m.p. 132°C
$[\alpha]_D^{18}$ =-181.7°	$[\alpha]_D^{19}$ =-182.9°

Unfortunately many crystallisations were necessary to obtain a pure product and a sample for deuterium analysis was not available.

26. The Examination of the Products Formed During an Attempted Deuteration of Cholesterol with a Platinum Black Catalyst.

This experiment was conducted on similar lines to those describing the deuteration and isomerisation of ergosterol.

Cholesterol, heavy water, acetic acid and a platinum black on asbestos catalyst were shaken together for three days at 125-129°C. The volatile components of the mixture were removed by distillation and the acetic acid converted to silver acetate. The solid residue, consisting of sterols and catalyst, was boiled with alcohol and the catalyst filtered off.

The sterol residues were then chromatographed and thereby divided into some twenty five fractions. No.4 yielded a crystalline sample of cholesteryl acetate m.p. 114.5°C, $[\alpha]_D^{19} = -40.5^\circ$ ($CHCl_3$). Sample 14 was apparently cholesterol itself, m.p. 147.5°C $[\alpha]_D^{20} = -40.0^\circ$ ($CHCl_3$). Between these two fractions lay six fractions of similar crystalline appearance - that of elongated thin plates.

Five of them melted between 77° and 80°C and one at 72°C. Measurement of the optical rotation of two samples yielded the following results:-

No.8 $[\alpha]_D^{19}$ + 86.7°

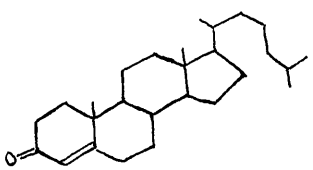
No.9 $[\alpha]_D^{19}$ + 82.4°.

These results were significant for all natural sterols which having a 5-6 double bond are laevorotatory and chol-

esterol isomers with the double bonds in △ 8, △ 8 (14) △ 14 and △ 4 positions all have positive rotations.

It seemed possible that the products might have been molecular mixtures of △ 4 cholesten-3 ∝ ol and △ 4 cholesten-3 β ol. This molecular mixture melts, however, at 141°C and furthermore the Rosenheim colour reaction expected of a △ 4 cholesten-3 ol sterol was negative (39).

The characteristics of these chromatograph products agreed however with those recorded for △ 4 cholesten-3one.



Product	△4 Cholesten-3one.
m.p. 78.5°	m.p. 79-80°, 81-82°
$[\alpha]_D^{19}$ +86.7° ($CHCl_3$)	$[\alpha]_D$ +83.5° +88.6°
$\lambda_{max.}^{ether}$ 234 mμ.	$\lambda_{max.}^{ether}$ 234 mμ.
semicarbazone	semicarbazone
m.p. 231° (dec.)	m.p. 234° (dec.)

Analysis - found %
C, 76.09: H, 10.54: N, 9.66.
calc. %
C, 76.14: H, 10.73: N, 9.51.

Deuterium Contents

Cholesterol Atom % D = .12

△ 4:cholesten-3-one Atom %D= .21.

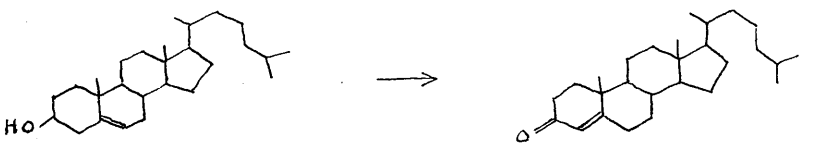
The function of the catalyst in producing the 3 ketonic group from the 3 hydroxy group should not be overlooked in addition to its function in introducing deuterium atoms into the parent sterol and producing double bond migration.

It seemed not unlikely at this stage to suggest that the deuterium atoms of deuterio cholesterol had been introduced into the 3 α hydrogen positions and into positions 4 and 7.

In the case of ergosterol deuteration with a platinum on asbestos catalyst no unchanged ergosterol was recovered and its absorption maximum was not detected in the absorption curves. In the deuteration of cholesterol under the same conditions both sterol and isomer have been isolated.

An Investigation of the Positions 3 and 4 in Deuterio Cholesterol in an Attempt to Locate Deuterium Atoms.

A conversion has been made of deuterio cholesterol to Δ 4 cholesten-3-one to determine the number of deuterium atoms occupying positions at carbon atoms 3 and 4.



The means employed were those which seemed least likely to cause exchange of deuterium atoms and hydrogen atoms in other parts of the molecule.

The reactions involved were:-

a. Bromination of deuterio cholesterol to the dibromide.(45).

b. Oxidation of the cholesterol dibromide to 5,6-dibromo-cholestan-3-one by chromium trioxide in acetic acid at room temperature.(46).

c. Recovery of the dibromide and zinc dust debromination in alcoholic solution acidified with a few drops dilute acid to produce Δ 4-cholesten-3-one.(47).

Results.

Atom % deuterium in deuterio cholesterol	=	0.12 %
Atom % deuterium in Δ 4-cholesten-3-one	=	0.12 %

From this result it must be concluded that the deuterium atoms in the deuterio cholesterol do not occupy positions on carbon atoms 3 and 4.

27. Part IV. The Role of the Solvent in Deuterium Exchange Reactions.

In all the deuteration experiments mentioned previously, the "solvent" employed has consisted of heavy water and acetic acid.

The role of the acid in sterol exchange reactions has been investigated by two methods. First, an attempt was made to answer the question: "Is another acid efficacious in bringing about exchange?" Secondly, to decide whether or not deuterio acetic acid is an intermediary in the deuteration of sterols, an experiment was conducted involving an attempted exchange between a sterol and anhydrous deuterio acetic acid, with the deuterium atoms in the methyl group.

28. Part IVA. Deuteration of Cholesterol using Dilute Sulphuric Acid and Platinum Black as Catalysts.

The following table shows the reaction mixtures used in attempted deuterations of cholesterol with the results.

Table XVIII.

Sterol	Heavy Water	Acid	Platinum Catalyst	Reference	Exchang
x	x			1	Nil
x	x	Sulphuric Acid		31,32	Nil
x	x		x	1	Nil
x	x	CH_3COOH	x	1	Exchange

A combination missing from the table was sterol, heavy water, sulphuric acid, platinum catalyst.

A deuteration of cholesterol was attempted on these lines. A sample of cholesterol melting at 148.5-149°C, $[\alpha]_D^{20}$ -39.5° was refluxed for 26 hours in alcohol containing heavy water and dilute sulphuric acid. The catalyst used was Adams' Platinum Black catalyst.

The sterol was recovered and the crystalline product, m.p. 148°C, dried and an estimation made of its deuterium content.

The % D_2O in the water obtained was 0.02%, c.f., ordinary water 0.017%.

It may be concluded that either the reaction temperature was not sufficiently high to cause deuterium exchange or that the normal acetic acid solvent has a peculiar role to play.

29. Part IVB. The Role of the Acetic Acid Solvent.

Bloch and Rittenberg (1) had shown that the deuteration of cholesterol with heavy water alone was unsuccessful even at elevated temperatures. The addition of both acetic acid and a platinum catalyst proved efficacious in making deuteric cholesterol.

The work described in Part I had shown that the solvent itself was deuterated by a process which is not independent of the nature of the sterol present, and unpublished work in the department (J.M. Turnbull) showed that acetic acid could be deuterated alone by a platinum catalysed reaction with heavy water.

The shape of the graphs obtained from a s tudy of the dependence of the extent of deuteration on the quantity of catalyst present seemed to suggest, when considered with these relevant facts, that the deuteration of the sterol proceeded by means of a mechanism involving consecutive simultaneous reactions:-

$$D_2O + CH_3COOH \underset{Pt}{\overset{I}{\rightleftharpoons}} HOD + \underbrace{CH_2D\,COOH}$$

$$+$$

$$\text{Sterol}$$

$$\Downarrow \text{ II} \quad Pt$$

$$CH_3COOH$$

$$+$$

Deuteric sterol.

Two separate stages were suggested - I in which deuteric acetic acid is formed, and II the reaction of this intermediary with sterol to produce a deuterio sterol.

If this mechanism is correct, then it should be possible to detect an exchange reaction between a sterol and anhydrous deuteric acetic acid. This theory was examined by preparing pure deuteric glacial acetic acid, with the deuterium atoms in the methyl group only, and shaking this acid with a sterol and a platinum catalyst for 3 days at 123°C - 133°C. To prepare the acid, attempts were made by distilling silver deuteric acetate with sulphuric acid or phosphoric acid, but the product was invariably moist. The acetic acid produced melted always below 13°C (c.f., 16.8°C for pure acid).

Finally, a method was adopted whereby dry silver deuteric acetate was treated with phosphoric acid and the resultant acetic and water mixture treated with triacetyl borate, refluxed and distilled, the water being removed by the following reaction:

$$B(OCOCH_3)_3 + 3\ HOH \longrightarrow B(OH)_3 \downarrow + 3\ CH_3COOH.$$

This meant that the deuterio acetic acid in the aqueous mixture was diluted with normal acetic acid to some extent.

The acid thus produced melted sharply at 16.8°C. Some of this acid was converted to silver acetate in order to estimate the methyl group deuterium content and the remainder was placed in a " Pyrex" reaction tube with some purified

ergosterol and a platinum catalist - Platinum Black.

The contents of the tube were frozen in acetone and solid carbon dioxide and the tube evacuated and sealed, shaken for three days at 123°C - 133°C and the sterol isolated. Considerable change in the sterol had taken place and difficulty was found in isolating a pure sample.

An analysis of this product however showed that only a very slight exchange of deuterium atoms had taken place - negligible compared with that found before in previous deuterations of sterols. There was little change in deuterium content of the acetic acid before and after the experiment. The slight deuterium exchange detected was due probably to the presence of a little water from the formation of the steryl acetate. These experimental results, of which details follow, led to the conclusion that the theory of consecutive simultaneous reactions was without foundation. Probably the deuteration takes place by means of simultaneous side reactions and the presence of the acetic acid is perhaps required to provide H^+ ions.

Constituents of Reaction Mixture.

Ergosterol	.7920 gm.
Platinum black on asbestos	.6220 gm.
Deuteric acetic acid	3.1650 gm.

Results

Table XIX

Atom %D in Ac'		Atom % D in sterol product
initially	finally	
1.65	1.63	.17

30. Part IVC. The Role of the Platinum Catalyst in the Deuterium Exchange Reaction.

In trying to elucidate the role of the catalyst in influencing the course of an exchange reaction between a substance containing hydrogen, and heavy water, an attempt was made to find an analogy between the operation of the catalyst and that of other deuterating agents.

In the first place similarities between platinum catalysts and acid or alkaline agents are seen in the following reactions:-

(1) 77 mols. % sulphuric acid produced exchange between heavy water and n-hexane and methylcyclohexane (11).

(2) A similar result for n-hexane has been reported in Part II using a platinum catalyst.

(3) Benzene exchanges with heavy water under the influence of both a platinum catalyst (4,12,13) and sulphuric acid (14,12).

(4) Acetic acid exchanges with heavy water in acid or alkali (15) or with a platinum catalyst (unpublished work by J.M. Turnbull of this department).

A few of the dissimilarities between the action of platinum catalysts and acid deuterating agents are illustrated in the following reactions:

(5) In the case of palmitic acid deuterium sulphate produces 'α' hydrogen exchange only, whereas active platinum produced deuterium exchange throughout the chain length of the acid.

Alkali insufficient to neutralise the acid was to be present in the latter case.

(6) Although cyclohexane was resistant to deuterium exchange even in the presence of deuterium sulphate a platinum catalyst produced 30% - 40% substitution of deuterium for hydrogen (11).

An analogy suggested by the "random" deuterium distribution in 4 above was between platinum catalysed exchange and exchange due to a free radicle mechanism. This was investigated therefore as follows.

An inorganic reaction believed to proceed by means of such a mechanism is that between ferrous sulphate and hydrogen peroxide. Haber and Weiss (16) suggested that the free radicles were produced as follows:-

$$Fe'' + HOH \rightleftharpoons Fe''' + {}^{\cdot}OH + (HO:)^{-}$$

This decomposition was conducted in an aqueous acetic acid, heavy water cyclohexane mixture and the hydrocarbon and acetic acid afterwards isolated and their deuterium contents examined. There was no exchange detected within the limits of the method of estimation used.

An organic substance reputed to be a producer of free radicles is dibenzoyl peroxide.

$$\phi COO - OOC\phi \rightarrow \phi COO^{\cdot} \rightarrow \phi^{\cdot} \rightarrow \phi - \phi + \phi COO\phi + \phi COOH.$$

This substance was prepared and decomposed in a mixture of acetic acid, heavy water and cyclohexane. Again no deuterium

in excess of the normal was found in the isolated constituents of the reaction mixture. At least in the case of cyclohexane and the methyl group of the acetic acid, it was concluded that the platinum catalyst does not operate by means of such simple free radicle decompositions.

31. Observations on the Mechanism of the Exchanges.

The consideration of the mechanism involved in these exchange reactions will be divided into several parts for it seemed likely that in the deuteration of the series of hydrocarbons and during the deuteration of the sterols themselves, some or all of these processes may have been operating.

The analogies are drawn with experiments in which the exchange occurred with deuterium gas and the mechanism of exchange may be different. Polanyi (4) found that whereas benzene exchanged more rapidly than saturated hydrocarbons with deuterium gas, the rate for water was about the same.

1. Exchange Reactions of Saturated Hydrocarbons.

D.D. Eley in a review of the "Mechanism of Hydrogen Catalysis" (36) suggests that a dehydrogenation equilibrium of the type $C_6H_{12} \rightleftharpoons C_6H_6 + 3H_2$, would lead to an exchange of deuterium atoms: such a reaction is much too slow to account for observed exchanges.

The review contains a more likely chemisorption of the type

$$C_nH_{2n+2} \rightleftharpoons (C_nH_{2n+1})M + HM$$

M - metal atoms of the catalyst.

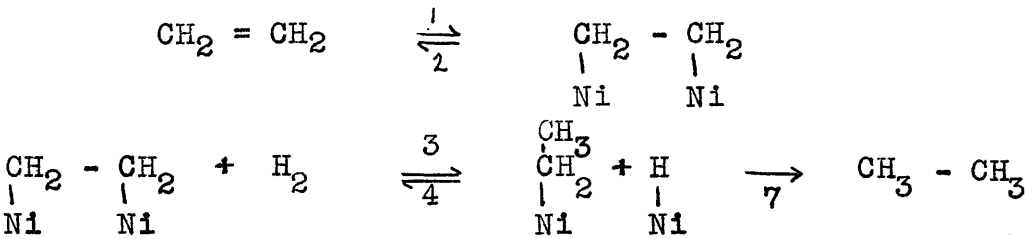
The high deuterium content of the competing methyl group of the acetic acid found in the experiments in the deuteration of normal and cyclohexane is evidence of the slight influence such a mechanism must have had on the deuteration of saturated

hydrocarbons.

Where the competition for the catalyst surface increased as in the case of cyclenes or alkenes, so also did the deuterium content of the acetic acid decrease.

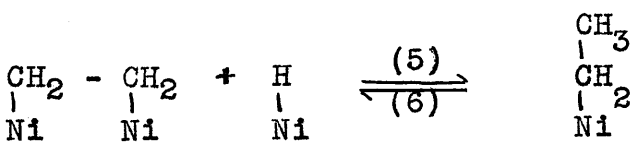
2. Reactions of Unsaturated Hydrocarbons.

G.H. Twigg in a report of the gaseous hydrogenation and exchange of ethylene (37) sets forth various reactions which account for the experimental observations.



The hydrogen molecules are van der Waal's adsorbed molecules.

He also adds a reaction



and these reactions satisfy all the features of exchange and hydrogenation.

In the deuteration of olefines and unsaturated sterols steps 4 and 2 or 6 and 2 would account for deuteration (cf., amount of deuterium found in these positions in deuterio cyclohexene). Step 7 might begin to account for the disproportionation reactions.

3. The Hydrogen Switch Mechanism.

Related, no doubt, to the preceeding mechanism is the "Hydrogen Switch Mechanism". In this mechanism, suggested by Turkevich and Smith (38), the catalysts, liquid or solid, were formulated as configurations of atoms with a hydrogen acceptor and donor at a distance of about 3.5Å.

For butene

$$\underset{\text{catalyst}}{CH_2 = CH - CH_2 - CH_3} \longrightarrow \underset{\text{catalyst}}{CH_3 - CH * CH - CH_3}$$

Such a mechanism would account for the shift of the double bonds of ergosterol and cholesterol and for the deuterium distribution in the ring of the deuterio cyclohexene.

This might be made more closely analogous to the exchange of ethylenic compounds by suggesting

$$CH_2 = CH - CH_2 - CH_3 \rightleftharpoons \underset{M\quad M\quad M}{CH_2 - CH - CH_2 - CH_3}$$

$$\underset{M\quad M\quad M\quad M}{D\ CH_2 - CH - CH_2 - CH_3} \qquad \underset{M\quad M\quad M}{CDH_2 - CH - CH_2 - CH_3}$$

$$CDH = CH - CH_2 - CH_3 \qquad CDH_2 - CH = CH - CH_3$$

$$MH\quad M\quad M \qquad M\quad M\quad MH$$

However it is formulated it seems that this hydrogen switch mechanism is operating and it can be used to account both for deuteration and isomerisation.

Appendix I. Experimental Details of the Experiments Described in the Thesis.

App.I 3. The Deuteration of Ergosterol and Cholesterol by a Platinum Catalysed Exchange Reaction.

Method.

Acetic acid, heavy water and platinum oxide in the quantities shown in Table I were weighed into 6" x ½" test tubes. Hydrogen was then passed into the suspension for 90 minutes to reduce the platinum oxide. This was followed by nitrogen for 60 minutes to remove as much of the hydrogen as possible. Weighed quantities of the sterols were added and the contents of the tubes were frozen in an acetone, solid carbon dioxide mixture. The tubes were then evacuated and sealed. In each experiment two tubes were prepared, one containing cholesterol, the other ergosterol and the two tubes were then shaken side by side for three days at a temperature of 123° to 133°C. The contents of the tubes were frozen as before and the tubes opened. The acetic acid and water from the reaction mixture were distilled off under vacuum.

Recovery of Sterols.

The residues from the distillation consisted of platinum, sterol, steryl acetate and decomposition products. Ether was added and the suspension filtered leaving behind the platinum. The filtrate was evaporated to dryness and the sterol acetates hydrolysed with alcoholic potash. In the case of cholesteryl acetate this was done by refluxing for

40 minutes with 40 ml. 2% solution and with 60 ml. 5% solution for ergosteryl acetate (17).

Some of the alcohol was then removed by boiling under reduced pressure and the remainder by evaporation at 50°C.

The solids remaining consisted of sterol, decomposition products, potassium hydroxide and potassium acetate.

Extraction with ether and water now proved effective and the ether layer was evaporated to dryness to give a reddish solid contaminated with oil and tar. Repeated recrystallisations from alcohol, benzene-alcohol mixtures and acetone produced crystalline sterols. In later experiments this wasteful process was superseded by chromatographic separation, as only 0.8 gm. of sterol was used in each reaction compared with the 12 gm. used by Bloch and Rittenberg.

For the chromatographic purification of the deuterio sterols a column measuring 15 cm. x 1 cm. was used. B.D.H. aluminium oxide for chromatographic analysis was heated for 30 minutes at 130°C and added to the column in pure dry benzene suspension. The solids from the ether extraction were dissolved in about 35 cc. 60° - 80° petroleum ether and allowed to drip slowly through the column at atmospheric pressure.

Ergosterol Chromatography. Petroleum ether elution produced firstly a yellowish red oil. Further elution with the same solvent and then with benzene yielded the sterol.

The sterol fractions were mixed and recrystallised three times from dry alcohol. A sample melted at 160°C in vacuum and mixed m.p. in vacuum with ergosterol (m.p. 163.5°) was 163°. The melting points of other samples, purified by recrystallisation only are shown in Table II.

Cholesterol Chromatography.

Petroleum ether was again used as solvent and for elution. Fractions were collected every 20 ml. passing through the column and at first these yielded brown oils. The fractions following yielded sterols and further sterols were eluted with 1:1 benzene petroleum ether.

The cholesterol was crystallised from alcohol.

Recovery of Acetic Acid.

The distillate consisted of water and acetic acid and perhaps some decomposition products of the reaction. This liquid was added to a 30% excess of silver oxide, a quantity known to ensure 99.9% conversion of the acetic acid present to silver acetate. The silver acetate was extracted with hot water and the silver oxide filtered off. The acetate was recovered by crystallisation and dried, even though some slight decomposition took place, at 130°C for 40 minutes.

By this means only water from the carbon bound hydrogen and deuterium was collected as the carboxyl hydrogen or deuterium was replaced by silver.

This method of recovering the silver acetate was liable to produce some exchange of the deuterium atoms of the methyl group. It was necessary therefore to ascertain the extent of this exchange, if any.

That exchange was negligible in the silver acetate recovery was shown as follows:

2.5 gm. deuterio silver acetate were boiled in 400 ml. water together with 1 gm. silver oxide. The suspension was filtered after boiling for 30 minutes and the silver acetate solution evaporated down over the course of a day. The crystals collected were dried and their deuterium content estimated.

$\%D_2O$ in water from combustion of original acetate = 10.64%

$\%D_2O$ in water from treated acetate = 10.76%

The method of recovering the acetates was considered to be satisfactory.

Preparation of Materials.

Acetic Acid. Analar acetic acid was refluxed twice for three hour periods with chromium trioxide, 1 gm. per 100 ml. acid. The acid was then distilled, the fraction boiling between 116-118°C collected and frozen to 15°C. The supernatant liquid was discarded leaving glacial acetic acid.

Water. Distilled water was distilled twice from potassium permanganate, then twice alone using an all glass apparatus, collecting the product in an 'aged' bottle.

Catalyst. Adams' catalyst was prepared for the experiments in Part I (19). The hydrogen for the reduction was prepared from arsenic free zinc. It was purified by passing it through aqueous alkaline permanganate, then silver sulphate solution and by drying in a caustic soda tower.

Heavy Water. "Norsk Hydro" deuterium oxide 99.6 gm./100 gm. d_4^{20}, 1.10496 was used.

Ergosterol. Glaxo Laboratories 85% Ergosterol was used as starting material. It was purified by the method of Bills and Cox (20), using 2:1 alcohol benzene mixtures for recrystallisation: the final product melted at 163.5°C in vacuum and $[\alpha]_{Hg}^{20}$ was -160.2° in 1% chloroform solution.

Cholesterol was extracted from gallstones with alcohol in a Soxhlet apparatus. Recrystallisations from alcohol gave a product m.p. 146-147°C.

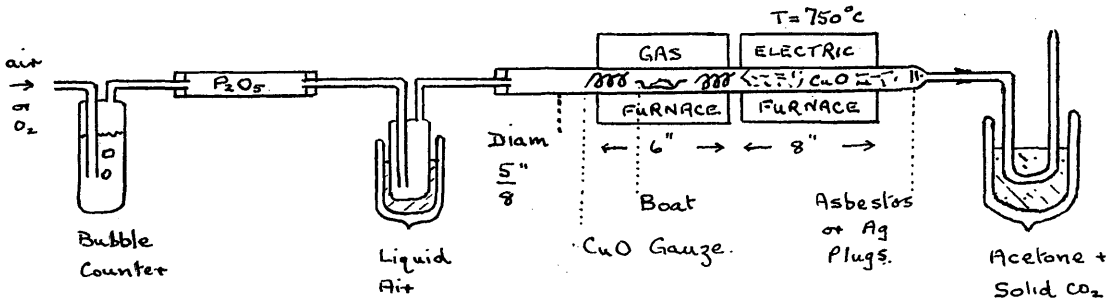
Benzene. After shaking with concentrated sulphuric acid to remove thiophen, the benzene was washed with aqueous alkali and water. This was dried with calcium chloride, cooled to +4°C and the solid collected, melted and distilled off sodium.

Ether. This was distilled off caustic potash, dried with calcium chloride, treated with sodium and distilled.

Alcohol. Lund and Bjerrum's method of purification was used (21).

Combustion of Deuterio Organic Compounds.

Fig.2. Combustion Apparatus

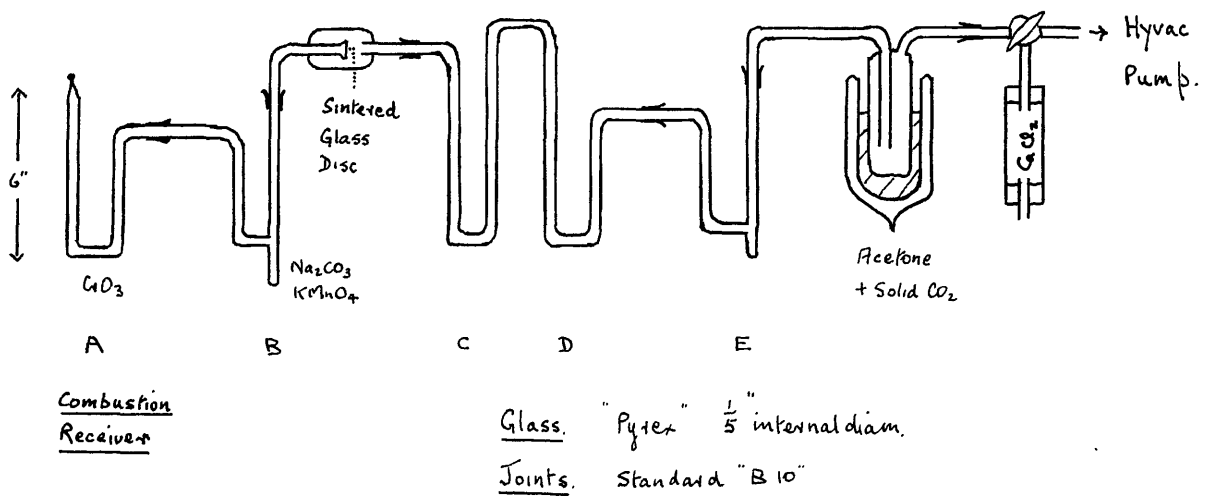


After thorough drying of the apparatus the receiver was immersed in an acetone, solid carbon dioxide freezing mixture and air passed through the apparatus for a test period. Provided the receiver was dry combustion was begun and the water produced collected. The amount of water required for the distillation which followed was from .03 to .08 gm. and of this, .01 gm. was required for deuterium estimation.

For the combustion of volatile substances an asbestos plug was inserted in the combustion tube to retard air flow and ensure efficient combustion. If halogen compounds were to be burned then silver wool was inserted.

Purification of the Water from the Combustion Process.

Fig.3. Distillation Apparatus.



Chromium trioxide freshly distilled in vacuum was added to the water which had been collected in receiver A, which was then warmed to 50° - 60°C. The open end of A was sealed and the water distilled under vacuum into the receiver B, immersed in a freezing mixture. B contained dry potassium permanganate and anhydrous sodium carbonate. To prevent these powders being swept throughout the apparatus on applying the vacuum pump, they were placed in the limb of B out of the flow of air. As a further precaution a sintered glass plate was fitted between B and C. When all the water had been distilled into B, air was admitted and the alkaline

permanganate solution refluxed for a moment or two with a small flame. After cooling, vacuum distillation was continued into C. From C the sample was distilled into D, collected finally in E and transferred to a glass capsule which was sealed, awaiting estimation of the deuterium oxide.

This apparatus is a modified version of that used by Keston, Rittenberg and Schoenheimer (18).

Before the combustion and purification processes were used on deuterio compounds, the methods were "proved" by taking a sample of heavy water, dividing it into two parts and subjecting one to the full combustion and distillation process. On estimation, the deuterium contents of the processed portion and the original portion differed by .04% deuterium oxide by weight.

Repetition of combustion, distillation and estimation of the deuterium content of organic compounds produced results differing by no more than .02% D_2O, w/w.

Estimation of the Heavy Water.

Dimensions in cm.

The "Gradient Tube" Method was used for theestimations (3). A glass tube of the dimensions shown was placed in a thermostat and a mixture of bromobenzene, xylene or bromobenzene, tetralin of density 1.02 at 25°C poured in until it was half full. A second mixture of the same components of density 0.99 was then layered over the first. The two liquids were then stirred together with a spiral stirrer, starting with short strokes at the junction and regularly increasing the amplitude of the strokes till the last was the length of the tube between the reservoirs. By this means a linear density gradient was obtained. The number of strokes used was twenty (one stroke upwards and one downwards counted as two strokes).

After standing 48 hours the gradient was calibrated by dropping in standard drops of known deuterium content. These came to rest at their own density level. They drifted slowly at the rate of .001 cm. in 20 minutes. The height of the drops above an arbitrary level was measured 20 minutes

after insertion.

The drops of unknown density were then inserted and their heights measured with a cathetometer calibrated to read .001 cm. From a graph prepared by the calibration process, the deuterium content of the samples could be determined.

The gradient length was usually about 9 cm. covering 0 - 14% D_2O, w/w, with 14 standard drops. That the gradient was zero across the tube was shown by inserting drops of the same heavy water on different sides of the tube. These came to rest at the same height.

After measurements were completed the drops of heavy water were removed from the tube by a long thin glass rod with a small piece of moistened filter paper rolled round one end. This was dipped carefully into the gradient tube and the drops absorbed on the filter paper. After standing 12 hours the tube was ready for further use.

App.I, 5. Deuteration of a Series of Hydrocarbons and an Alcohol.

Procedure. The substances were prepared by the methods to be described, and then weighed into "Pyrex" brand reaction tubes. The quantities are shown in Table V.

After weighing the other constituents into the reaction tubes the tubes were cooled with acetone and carbon dioxide or liquid air. After the contents had frozen, the tubes were evacuated with a "Hyvac" pump and sealed. Four tubes at a time, comprising a set, were then shaken for three days at 123°C - 133°C. The hydrocarbons were then isolated and their deuterium content measured together with that of the acetate group of the solvent.

Experiments 1-4, 5-8, 9-12 constituted Group I.
All the experiments were repeated and
Experiments 17-20, 21-24, 25-28 constituted Group II.
The only difference between the groups was that the catalyst had aged before Group II was begun. This probably accounts for the decreased deuterium transfer in Group II.

Isolation of the Constituents of the Reaction Tubes.

The tubes were frozen as before and opened. The two layers of liquid were then separated and the hydrocarbons dried and distilled and the acetic acid water layer added to silver oxide: the silver acetate produced was isolated by crystallisation. In the case of the cyclohexanols, the

two liquid layers had merged and alcohol and the acid had to be separated by different means.

(a) Isolation of the Hydrocarbons.

The hydrocarbons were dried by chemical means and distilled. The drying agent used was solid "Analar" caustic soda. This served the double purpose of removing water and removing any acetic acid dissolved in the hydrocarbon, as sodium acetate. The hydrocarbons were allowed to stand for at least 24 hours at room temperature before distillation. In a few cases the hydrocarbons, when distilled, left a drop or two of a coloured liquid of boiling point higher than that of the hydrocarbon. It was assumed that these liquids were polymers formed during deuteration and they were collected in several cases and an analysis made of their deuterium contents.

(b) Isolation of the Cyclohexanol from expts. 3 and 24.

The cyclohexanol was probably present as cyclohexyl acetate.

Expt.3. The contents of the reaction tube, after removal of the catalyst were refluxed with 1 cc. concentrated sulphuric acid in 15 ml. water for 2 hours. A further quantity of acid 1 ml. conc. in 15 ml. water added and the refluxing continued for 40 minutes.

The solution was rendered alkaline with caustic soda and extracted with ether. The ether extract was dried over

potassium carbonate and the cyclohexanol recovered by distillation. The aqueous layer from the extraction was retained for the recovery of the acetic acid.

The cyclohexanol, however, smelled of ester and was therefore hydrolysed again with aqueous caustic soda. 3-4 ml. of the ester alcohol mixture were refluxed with 3 gm. caustic soda in 15 ml. water for 4 hours. The alcohol was then recovered as before, B.P. 158-160°C and no smell of ester apparent.

Expt.24. The homogeneous liquid from this reaction tube was filtered into a flask and refluxed for 3 hours with 3 gm. caustic soda in 25 ml. water. The alcohol was recovered by ether extraction and smelled only of cyclohexanol. The aqueous layer was retained as before.

(c). Isolation of $\Delta^{1:4}$ and $\Delta^{1:3}$ Cyclohexadienes, Expts.10 and 11, 18 and 19.

Lest the caustic soda drying should produce polymerisation, these hydrocarbons were isolated by washing the hydrocarbon layer with water, drying it over calcium chloride and distillation.

(d). Isolation of the Acetic Acid of the Reaction Mixtures as Silver Acetate.

The acetic acid water layer containing the platinum catalyst in suspension was washed on to 4 gm. silver oxide, 20 ml. water added and the mixture warmed on the steam bath

for a few minutes. The mixture was then diluted to 300 - 400 ml. and boiled. Whenever the solution boiled the unused oxide and catalyst were filtered off. The silver acetate solution was then evaporated down to about 250 ml. on the steam bath and filtered. Further evaporation then produced crystals of silver acetate which were collected and dried at 120°C - 125°C for 40 min. before combustion.

The acetic acid from the hydrolysis of the mixtures from the cyclohexanol tubes was left in aqueous solution, after ether extractions of the alcohol, as sodium acetate. This solution was acidified with concentrated sulphuric acid and the acetic acid recovered by steam distillation. The aqueous product was boiled with silver oxide, filtered and the silver acetate isolated as described above.

Preparation of the Components of the Reaction Tubes.

(a) Acetic Acid. 'Analar' acetic acid was purified by refluxing twice for three hour periods with chromium trioxide, 1 gm. per 100 ml. acid. The acid was then distilled, the fraction boiling between 116°C and 118°C being collected and frozen at 15°C. The supernatant liquid was discarded leaving glacial acetic acid.

(b) Heavy Water. "Norsk Hydro" deuterium oxide was used. The density and % by weight of the samples used were Expt. 1-8, 99.7%, 9-28, 99.75%.

(c) Hydrocarbons.

(i) Cyclohexane. Purified by distillation: fraction boiling at 80°C - 80.2°C collected.

(ii) n-hexane. Purified by distillation.

B.P. 68°C.

(iii) Cyclohexene. Purified by distillation.

B.P. 83°C - 83.5°C.

(iv) Cyclohexanol. Starting material was shaken with aqueous alkali to remove phenol, washed twice with water, dried over potassium carbonate and distilled. Fraction B.P. 161°C was collected.

(v) Pentene I. Prepared by method of Adams, Kamm and Marvel (22) by the dehydration of n-amyl alcohol with sulphuric acid. The product obtained was fractionated in a Widmer column and the fraction B.P. 38°C-39°C collected.

(vi) Octene I. A sample supplied by Messrs. Lights was used. This was distilled and fraction B.P. 119°-121°C used.

(vii) Octene II. Again a "Lights" sample was used, B.P. 122°-124.5°C.

(viii) $\Delta^{1:4}$ cyclohexadiene. This was prepared by hydrogenating hydroquinone to 1:4 ciscyclohexanediol using a Raney nickel catalyst and hydrogen under pressure. The diol was then dehydrated with concentrated sulphuric acid to yield the 1:4 diene.

Hydroquinone was recrystallised from water twice, after boiling with charcoal. Product was brownish in colour, M.P. 170°C.

Raney Nickel was prepared from a nickel aluminium alloy and caustic soda (23).

Hydrogenation of hydroquinone: the method described by Adkins and Cramer (24) was used, i.e., hydrogenation at 150°C at 170-130 atmospheres of hydrogen using Raney Nickel as catalyst. The solvent used was absolute alcohol previously distilled off a portion of the catalyst. The product had M.P. 101°-102°C. Conversion to the 1:4 diene was accomplished by distillation with sulphuric acid (25). Using a Widmer column, the fraction boiling at 87°C was collected.

(ix) $\Delta^{1:3}$ Cyclohexadiene. The preparation, attempted on similar lines to that for the $\Delta^{1:4}$ diene, yielded a substance which polymerised very readily on distillation in air. The diene used was prepared by the removal of hydrobromic acid

from 1:2 dibromocyclohexane with boiling quinoline.

1:2 dibromcyclohexane was prepared by brominating cyclohexene at less than -1°C in carbon tetrachloride solution (26) A clear liquid with slight brownish tinge was obtained, B.P. 98°C at 14-15 mm. This product was treated with freshly distilled quinoline and a sample of the 1:3 diene obtained, B.P. 83°-86°C (27).

(x) Benzene. "B.D.H." crystallisable benzene was treated with concentrated sulphuric acid to remove thiophen, shaken with aqueous caustic soda, washed with water three times and dried for three days over calcium chloride. The liquid was cooled in ice and the supernatant liquid decanted. The benzene left was melted and distilled. B.P. 80.0-80.5°C.

(d). A platinum black catalyst was used in these experiments. A preparation in bulk was made for all experiments cited in this thesis, except those in I, 5 using Adams' catalyst. Adams' catalyst was thought to be unsuitable although it had been used for the sterol exchange reactions. A preparation required for a series of comparable experiments could not be made of Adams' catalyst, nor could the catalyst be made under exactly the same conditions each time. The surface of Adams' may also be contaminated with hydrogen adsorbed during the preparation. The catalyst used was prepared by reduction of an aqueous solution of a platinum salt by formalin. Asbestos ("Gooch Asbestos") was used as carrier (28).

Bromine Absorption Values of Starting Materials.

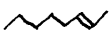
Expt. No.				
23,	25	cyclohexene	.94	Double Bonds per molecule
2,	22	cyclohexane	.01	" "
12,	20	benzene	.01	" "

App.I. 11. Values Abstracted from Table V Used in the Argument Section 11.

Reaction Mixtures and Results (expressed as % by weight D_2O in water from combustion of hydrocarbons and silver acetate). (wt. in gm. vol. in ml.).

Hydrocarbon	Catalyst	D_2O Wt.	$HA\bar{c}$	Hydrocarbon		% by wt. D_2O		Expt.
				Wt.	Vol.	Hydrocarbon	AgAc	
	.0810	.4328	1.653	2.8080	3.42	0.77	6.70	23
	.0810	.429	1.641	2.5665	3.85	2.96	12.63	6
	.0810	.4325	1.651	3.9765	5.57	2.21	10.64	7
	.0810	.4305	1.644	3.9760	5.52	1.96	9.41	8
	.0810	.4317	1.650	3.0010	3.6	0.66	7.59	25
	.0810	.4328	1.654	2.5630	3.85	1.39	7.84	26
	.0810	.4321	1.651	3.9770	5.57	1.73	7.32	27
	.0810	.4313	1.648	3.9730	5.52	1.37	8.94	28

Boiling Points of Hydrocarbons (in °C).

Expt.	Hydrocarbon	B.P. (°C)		Colour of Hydrocarbon		
		before deuteration	after deuteration	Starting Material	after deuteration	after purifying
23		83-83.5	81-84	Nil	Nil	Nil
25		83-84.5	81-84	Nil	yellow	Nil
6		38-39	38-39	Nil	Nil	Nil
26		38-39	37-39	Nil	wine red	Nil
7		119-121	120-122.5	Nil	pale yellow	Nil
27		120-121	120-122	Nil	pale yellow	Nil
8		122-124.5	123-124.5	Nil	Nil	Nil
28		123.5-125	122-124	Nil	pale yellow	Nil

App.I. 12. Experiments on the Conversion of Deuterio Cyclohexene to Adipic Acid and Bromadipic Acid.

Preparation of Deuterio Cyclohexene.

It was observed that attempted deuterations of cyclohexene which had been freshly distilled resulted in disproportionation of the cyclohexene. When the sample had aged and the double bond content had fallen to a value slightly less than 1 per molecule, the production of deuterio cyclohexene was successful.

The preparative method was that described previously in which .08 gm. platinum black on asbestos, .43 gm. 99% D_2O w/w., 1.65 gm. acetic acid, and 2.9 gm. cyclohexene containing .94 double bonds per molecule, were shaken for three days at 124-133°C.

The hydrocarbons were isolated and the following experiments conducted:

Expt. 1L.

Cyclohexene, B.P. 81°-84° containing 0.93 double bonds per molecule and having deuterium content equivalent to 0.92% D_2O was oxidised in "neutral" solution by aqueous potassium permanganate containing silver nitrate; hydroxyl ion concentration was thereby reduced by precipitation of silver oxide. It has been established that silver oxide did not cause deuterium exchange between methyl group hydrogen atoms in acetic acid and it was assumed that exchange in the case of the

adipic acid produced would be negligible. 0.510 gm. cyclohexene were placed in a flask under reflux condenser and 70 ml. of a warm solution containing 2.65 gm. potassium permanganate and 0.70 gm. silver nitrate was added. An immediate reaction ensued producing a brown precipitate. The suspension was boiled for 15 minutes and cooled. Boiling was then continued for a further 40 minutes. After cooling, the suspension was filtered and the colourless filtrate evaporated to about 60 ml. on the steam bath. A few drops of concentrated sulphuric acid served to acidify the liquid which was then extracted with ether. The ether extracts yielded a white solid which, on recrystallising from water, melted at 150°-151° and weighed .21 gm.

Expt. 2L.

.483 gm. cyclohexene, B.P. 82.8-84°C, containing .94 double bonds per molecule, was placed in a small flask and a solution of 1.6 ml. hydrogen peroxide (30%) in 7.1 ml. tertiary butyl alcohol added. After mixing, 0.1 ml. of 2% osmium tetroxide in tertiary butyl alcohol was added. A reaction started at once at room temperature and the colour produced in the solution varied from pale yellow to brown to wine red. After a few minutes the colour had disappeared and the flask was found to be warm. The reaction mixture was allowed to stand overnight at room temperature and the

solvents were then removed on the water bath. The residue was taken to be cyclohexane 1-2 diol. The diol was mixed with water and 1.8 gm. of potassium permanganate in 30 ml. water added. 3 hours later the suspension was filtered and the acidified filtrate extracted with ether. The extract was evaporated to dryness and the pale yellow solid produced recrystallised from water. Weight of yield .448 gm. The white product was then dried in vacuum over concentrated sulphuric acid before deuterium analysis. M.P. 150°-151°C.

Expt. 3L.

Cyclohexene, B.P. 81°-84°C containing .91 double bonds per molecule was oxidised by standing overnight at room temperature with 2.6 ml. 30% hydrogen peroxide and .13 ml. 2% osmium tetroxide in tertiary butyl alcohol as catalyst. As solvent for this mixture 10 ml. tertiary butyl alcohol was used. Weight of hydrocarbon at start of experiment was 0.650 gm. On mixing the reactants a yellow colour was produced but this had disappeared by the following morning. After removing the solvent, the residue was diluted and an aqueous solution containing 2.5 gm. potassium permanganate added. The suspension produced was filtered immediately and the permanganate in the filtrate discharged by acidifying and adding ferrous sulphate solution. Extraction many times with ether and evaporating the extracts to dryness produced a white solid which was recrystallised from water. Yield, 0.39 gm. M.P. 149-150°C.

Bromination of deuterio adipic acid from Expt. 3L.

.21 gm. of the adipic acid were refluxed with thionyl chloride (4 ml.) for 1.5 hours. After cooling, 0.17 ml. dry bromine were added and the solution refluxed for four hours. The following day, after adding excess formic acid, the flask was cooled in ice and a brown solid was filtered off. The solid was boiled with "Norit" charcoal and filtered. The pale yellow solid obtained by cooling the dilute solution melted at 182°-182.5°C. Many recrystallisations from formic acid failed to raise the melting point. The literature offers melting points of 139°C and 193°C for the "dl" and "meso" forms of dibromadipic acid and the product obtained in this experiment was taken to be a mixture of the two. As a check a sample of the acid was analysed for % bromine (Found: 52.88%. Calc. 52.57%). The acid, after drying in vacuum over solid caustic soda was almost white. Yield of pure acid, .17 gm.

Expt. 11A.

This experiment was carried out by Mr. Angus Macdonald of this department.

The technique used for the preparation of the deuterio cyclohexene was similar to that described already. Precautions were taken to isolate the cyclohexene from the deuteri product before combusion and deuterium estimation. Deuterium

in the heavy water due to the presence of deuterio disproportionation products was thereby eliminated. This preparation of cyclohexene free from contaminants was accomplished by brominating a portion of the products of deuteration and isolating the deuterio dibromcyclohexane. The method used is described in reference 42 and the dibromcyclohexane recovered by fractional distillation.

The conversion of deuterio cyclohexene to adipic acid was made by the method described in Expt. 3L. The preparation of deuterio dibromadipic acid was also that of Expt. 3L.

Expt. 12.A.

This experiment was also conducted by Mr. Macdonald on the same lines as outlined in Expt.11A.

The following data were recorded for the two experiments

		11A	12A
Double bond content of deuterio product		.840	.762
Oxidation method		OsO_4, H_2O_2	do.
Adipic Acid, m.p.		151°C.	151°C.
Dibromadipic Acid,			
Bromine Analysis	-- found	52.31%	52.97%.
	calc.	52.57%	
Dibromcyclohexane,			
Bromine Analysis	-- found	66.42%	
	calc.	66.04%.	

Sample Calculation, Expt. 3L.

Number deuterium atoms in one molecule cyclohexene = .0690

Number in one molecule adipic acid = .0594

Number in one molecule dibromadipic acid = $\frac{8x.062}{.062+11.04}$

= .0447

Number remaining in "α" position = .0594-.0447 = .0147

Total number in "α" position = 2 x .0147 = .0294

Number in "β" position = .0447 - .0147 = .0300

Number in CH:CH position = .0690 - (.0300+ .0294) = .0096

App.I, 15. Bromine Absorption Values of Hydrocarbons.

Standard bromine solution in carbon tetrachloride was added to a weighed quantity of the substance till a red coloration was produced. Aqueous potassium iodide solution was then added and the liberated iodine titrated with aqueous thiosulphate. The bromine solution was standardised during each set of experiments by running a measured quantity into aqueous potassium iodide and titrating the liberated iodine with thiosulphate.

The results were expressed as number of double bonds per gm. mol. of substance.

App.I. 18. Deuteration of Various Cyclohexene Samples.

Procedure. The reaction tubes were prepared and the specimens heated and shaken and isolated as in Experiments 1-28. Stocks of water, acetic acid, platinum catalyst as prepared for Experiments 1-28 were used as constituents of the reaction tube.

Constituents of Tubes and Results.

Expt. No.	Weights in gm.					% D_2O	
	Catalyst	H_2O	D_2O*	HAc	Hydrocarbon	Hydrocarbon	AgAa
29	.0810	nil	.3978	1.676	3.0565	1.04	8.53
30	.0810	nil	.4004	1.687	3.0000	10.13	1.52
31	.0810	nil	.3988	1.680	2.9900	10.12	1.77
32	.0810	.4635	nil	1.6600	3.0105	.04	.03

* % D_2O in heavy water at start 99.82% w/w.

App.I, 19.

Experimental details. Separation of isomerisation products of 1:4 cyclohexadiene.

The hydrocarbon mixture from expt. 18 was treated with 30% oleum. The reaction was allowed to proceed for 15 minutes at room temperature and a brownish colour developed in the solution. It was observed that some hydrocarbon floated on top of the mixture. The mixture was diluted with water and a greenish solution produced. The hydrocarbon floating on top was separated off, washed with water and dried with calcium chloride. This product was a clear liquid and was taken to be cyclohexane. The aqueous solution containing the sulphonated benzene was treated with boiling barium hydroxide solution till the solution was alkaline to litmus. The precipitate produced was filtered off and the benzene sulphonic acid isolated from the filtrate as the sodium salt. This was accomplished by adding sodium carbonate solution till no more barium carbonate was precipitated. The suspension was filtered and the filtrate concentrated. The crystals produced were collected and dried, and an estimate made of their deuterium content.

App.I, 23.

Experimental Details: Investigation of the Products in an Attempted Deuteration of Ergosterol.

Starting Materials.

Ergosterol. Messrs. Lights ergosterol was crystallised many times from a benzene alcohol mixture (1:2 by volume) till the product melted at 163.5°-164.8°C in vacuum.

$[\alpha]_{Hg}^{18}$ -163.5°.

Water: Acetic Acid. Stock solutions whose preparations were described in App.I, 3.

Platinum Black on Asbestos. Stock catalyst prepared in App.I, 5.

Heavy Water. "Norsk Hydro" 99.95% w/w D_2O and 99.75% w/w D_2O.

Reaction Vessel. "Pyrex" brand glass.

Weights of Reactants.

Catalyst	.8100 gm.	
D_2O	3.8800 gm.	99.95% w/w
D_2O	.2725 gm.	99.75% w/w
Water	1.3000 gm.	
Acetic Acid	17.3810 gm.	
Sterol	4.8035 gm.	

Tube and contents were frozen in a mixture of solid carbon dioxide and acetone. The tube was then evacuated and sealed and shaken for three days at 124-135°C. The tube was opened and the volatile components were removed by vacuum distillation at a temperature below 40°C. Total weight of volatile components recovered = 22.2910 gm.

Total weight of solvents at start of experiment = 22.8335 gm.
Weight of solvents left in sterol tube = .5425 gm.
It was assumed that some of the sterol had been converted to steryl acetate.

Removal of Catalyst. This was accomplished by dissolving the sterol residues in chloroform and filtering off the catalyst. Recovery of catalyst was quantitative.

Recovery of Sterol, etc. Slightly more of the solid matter was recovered than was expected. This may have been due to slight absorption of the chloroform in which the sterol and residues were very soluble.

Chromatographic Analysis. A column was prepared of "B.D.H." Alumina ("For Chromatographic Adsorption"). The sterol residues in benzene were added after wetting the column with "60-80" Pet. Ether. Elution was continued using pet. ether, benzene, chloroform, alcohol and finally acetone.

The colours of the eluted solutions ranged from dark brown to pale yellow. 5.5735 gm. solid were recovered compared with 5.3460 gm., the expected value. The excess weight was attributed to solvents retained in the gums and oils from the chromatograph. Each fraction from the chromatographic analysis, after evaporating to dryness, was dissolved in alcohol. These solutions were boiled with a little "Norit" charcoal and filtered. The products would not crystallise though many solvents and mixtures were tried for crystallisation. Sample No.10 eventually yielded crystals

from a mixture of alcohol and water. The investigation of the nature of these crystals is described later.

The other fractions from the chromatograph were seeded with a crystal or two from No.10. They were cooled in ice, in ice and salt, in a freezing mixture of acetone and carbon dioxide. The solutions were cooled slowly and quickly, evaporated down on the steam bath, their volumes reduced in dessicators at room temperature, all without success.

Investigation of the nature of crystals from Chromatograph Fraction 10.

These white crystals, m.p. 131°C were recrystallised twice from a mixture of benzene and alcohol (1:4 by volume). The melting point had risen to 135-136.5°C. They appeared to consist of needles but closer examination showed that they were in fact composed of elongated plates.

Weight of pure crystals obtained 0.05 gm.

Rotation $[\alpha]_D^{18}$ -50.9 in chloroform

.0345 gm. solid in 5 ml. solution.

1 ml. of this solution was diluted to 100 ml. with chloroform and examined in a "Unicam Absorption Spectrophotometer" using U.V. light from a hydrogen lamp. The absorption curve obtained is shown in Fig.5.

Examination of Fraction No.15 from the chromatographic analysis

This sample was rechromatographed in an alumina column as before and the same oils and gums obtained together with a

few crystals not yet examined.

Examination of those fractions which did not yield crystalline solids.

Since it appeared that some ergosteryl B1 acetate had been formed in the course of the deuteration experiment, it was decided to investigate the possibility of the formation of the isomers B2 and B3. The separation method developed by Windaus and his associates was used. A schematic diagram of the separation follows.

Ergosterols B1, B2, B3.

crystallisation from alcohol
yields solid. Filter ---------- Ergosterol B1

Filtrate
Treat with maleic anhydride
Extract with ether.

Soluble portion	Insoluble in ether
Ergosterol B2	Adduct maleic anhydride with Ergosterol B3.

The fractions from the chromatograph analysis, with the exception of Nos.10 and 15, were united and an attempt made to obtain crystals of the B2 acetate by crystallisation from a mixture of methyl alcohol and ethyl acetate. No crystals were obtained.

The solvents were removed and the residues dissolved in dry benzene and refluxed for eight hours with maleic anhydride

(1.56 gm. residue to 0.7 gm. maleic anhydride). The benzene was removed and 1.2 gm. potassium hydroxide in methyl alcohol added. The solution was boiled for a few moments and allowed to cool. The methyl alcohol was removed under reduced pressure and the residue diluted with water. 60-80° petroleum ether was used for the extraction of that material which had not reacted with the maleic anhydride. No residues were obtained from the petroleum ether extract.

The aqueous solution remaining after the extraction was acidified with concentrated hydrochloric acid. A whitish precipitate was produced and this was extracted with ether and the ether evaporated off. The residue was purified by dissolving the residue in aqueous potassium hydroxide and re-precipitating with hydrochloric acid. The product was dried in a vacuum desiccator and fruitless attempts made to re-crystallise it from acetic acid. Methyl alcohol, acetone, ethyl ether, ethyl alcohol, petroleum ether, dioxane and water, acetic acid and water were also tried.

Purification was effected by dissolving the substance in a large volume of acetic acid and pouring the solution into a large volume of water. Repetition of the process produced a brownish yellow solid, m.p. 146.5-150°C.

An analysis of this product yielded the results

Found: C, 68.92%, H, 8.68%

(Calc. C, 75.2%, H, 9.39% for adduct).

Fig.4. Ultraviolet Absorption Curve of Chromatograph Fractions 11, 21, 29. (Chloroform Solution).

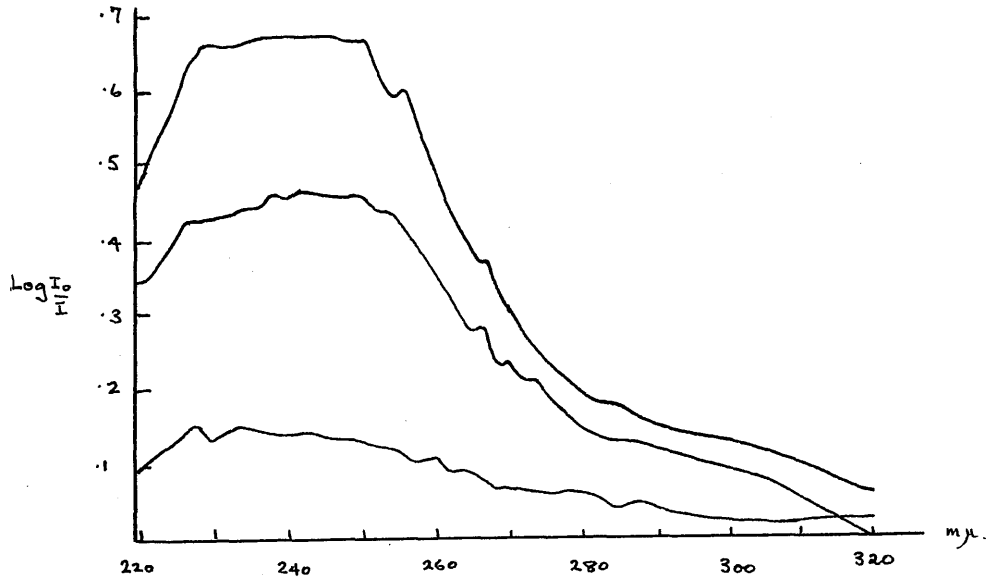
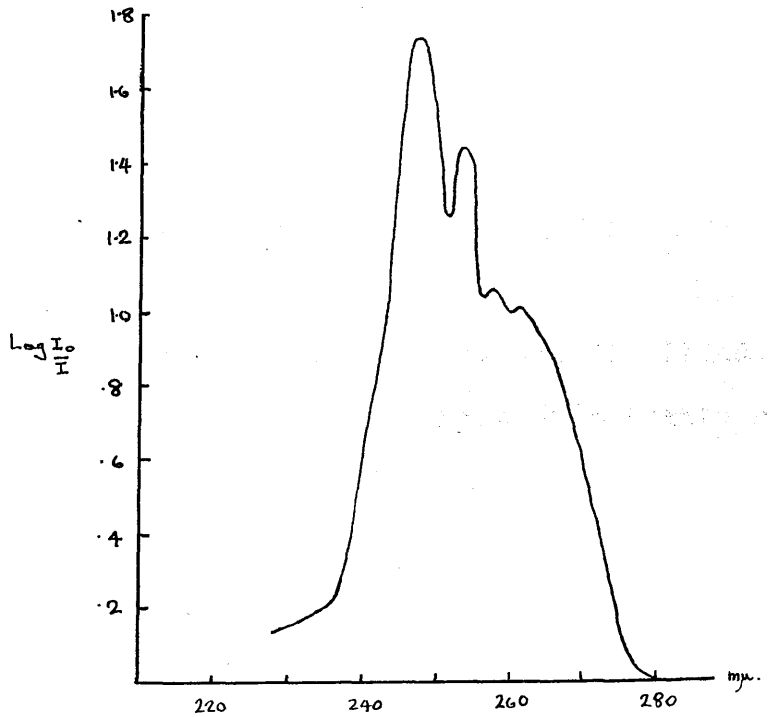


Fig.5. Ultraviolet Absorption Curve of Chromatograph Fraction 10. 0.0690 gm. in 1000 ml. Chloroform Solution.



App. I. 24.

Experimental Details. Deuteration and Isomerisation of Ergosterol.

Preparation of Ergosterol. 10 gm. "Lights" and 1.5 gm. "Glaxo" ergosterol were crystallised from a mixture of alcohol and benzene (2:1 by volume). The solution was boiled for a moment or two with charcoal during the first crystallisation only. Several crystallisations yielded 5.5 gm. of ergosterol, m.p. 163.5°C $[\alpha]^{19}_{Hg}$ -166.4° in chloroform. This product was dried at 85°C for 25 minutes before use.

Heavy Water. "Norsk Hydro" 99.57% w/w d_4^{20} 1.10496.

Water, Acetic Acid, Platinum on Asbestos. Samples were taken from the stocks whose preparations were described previously in App.I, 3 and 5.

Constituents of Reaction Tube (Expt. E7).

Weight of	Catalyst	0.8100 gm.
	Heavy Water	4.2800 gm.
	Water	1.2950 gm.
	Acetic Acid	17.2295 gm.
	Ergosterol	5.4330 gm.

The tube was evacuated and sealed as usual and shaken for three days at 125-129°C.

Recovery of Solvents. Solvents distilled off at 19 mm. pressure and 30-35°C. Weight of solvents recovered was 21.5 gm.

Weight of solvents at start 22.8125 gm.

Appearance of Tube on removal from Shaker.

1. Bottom of tube ---- grey catalyst
2. Liquid in tube clear and colourless.

3. Dark greenish solid or oil floating at top of liquid.

Recovery of Solids. The solids were washed out of the reaction vessel with hot alcohol. The solids were then boiled with charcoal in 200 ml. alcohol and filtered hot. The filtered solution was pale brownish yellow in colour.

Weight of catalyst and charcoal recovered, 1.0 gm.

The alcoholic solution was then seeded with a minute crystal of ergosterol and with another crystal of ergosteryl Bl acetate.

After standing overnight in a refrigerator a crop of pale green crystals was collected (Sample A). By reducing the filtrate on the steam bath and cooling the liquids in the refrigerator, two further crops were collected leaving a wine coloured filtrate.

Sample A	m.p.	128-131°C
Sample B	m.p.	125°C
Sample C	m.p.	124.5°C.

These samples were then combined and recrystallised many times from alcohol.

The physical characteristics of the final product suggested that this product was Ergosteryl Bl Acetate.

(a) C.H. analysis Found C, 82.32%, H, 11.17%
Calc. C, 82.13%, H, 10.57%

(b) λ_{max}^{ether} = 249 mμ. fig.6.

(c) $[\alpha]_D^{18.5}$ = -56.1°

(d) m.p. 139.5-141°C.

Fig.6. U.V. Absorption Spectrum of Product, E7.
(.00114 gm. in 125 ml. Ether Solution).

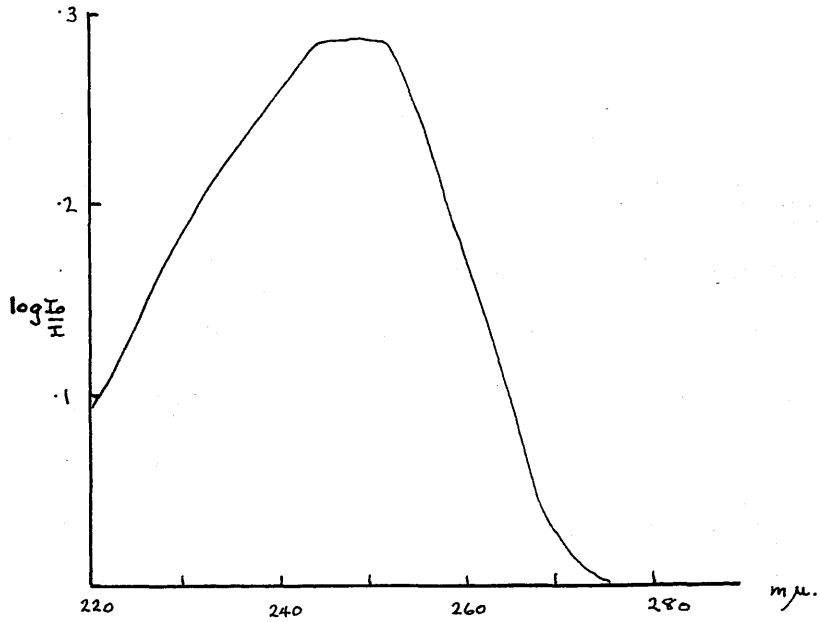
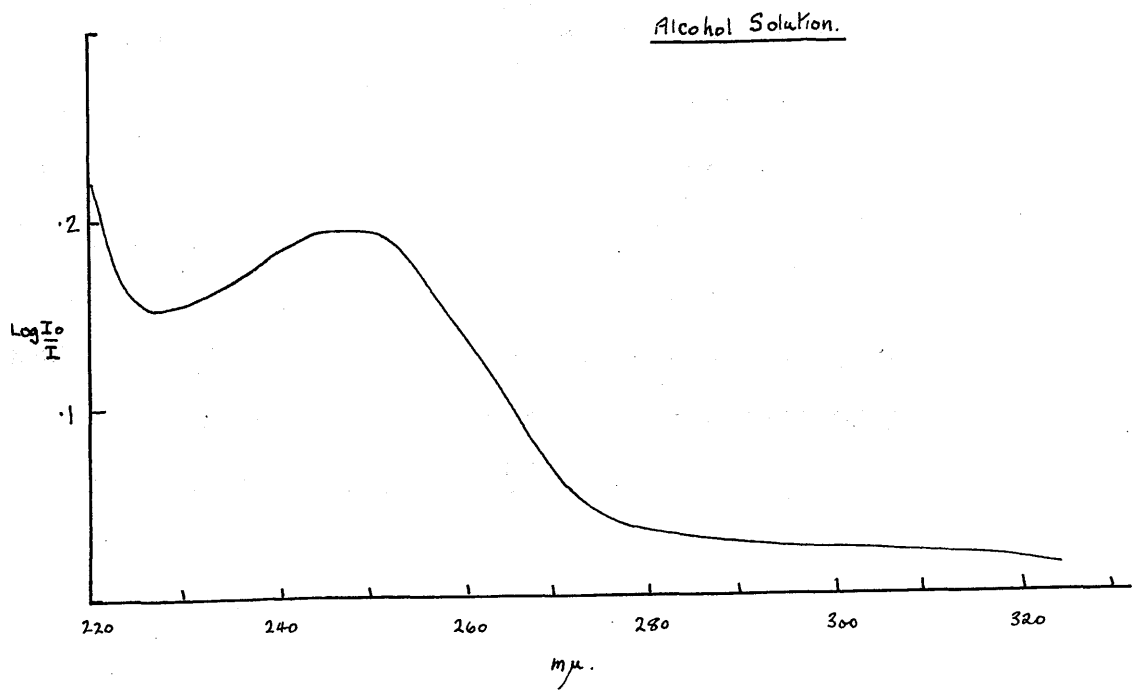


Fig.7. U.V. Absorption Curve, Residues E7.



Deuterium Analysis.

$\%D_2O$ in water from combustion of ergosteryl B1 acetate = 4.59%

$\%D_2O$ in water from combustion of silver acetate prepared from solvents used in the deuteration = 12.14%.

App.I.25.

Isomerisation of Deuterio Ergosteryl B1 Acetate from Expt. E7, using Dry Hydrogen Chloride Gas.

This isomerisation has been studied by Windaus et al.(10). When ergosteryl B1 acetate was treated with dry hydrogen chloride gas at 0°C, ergosteryl B3 acetate was produced. The isolated product was crystallised by Windaus from acetic acid. This process was forbidden in this experiment because of the possibility of exchange between deuterio acetate or acetyl groups and their normal equivalents.

Procedure:

Chloroform. The solvent was dried by means of calcium chloride after shaking with concentrated sulphuric acid and washing with water. The chloroform was distilled before use.

Dry Hydrogen Chloride Gas. This was obtained from a generator in which concentrated hydrochloric and sulphuric acids were mixed. The sulphuric acid, besides generating the gas, acted as a drying agent. The gas was cooled by passing it through a "U" tube immersed in ice.

The Ergosteryl B1 Acetate was dried for 15 minutes at 80°C. 0.22 gm. were dissolved in 4 ml. chloroform. The solution was cooled to 0°C during the reaction by immersing the containing vessel in ice.

Reaction. A stream of gas was passed through the solution from a capillary tube for one hour. The colour of the solution changed from brown at the start to blue tinged with pink at the end.

Recovery of Sterol. The chloroform solution was poured into a sodium bicarbonate solution at 0°C (1.5 gm. in 20 ml. water). The chloroform solution turned pale green. After shaking the chloroform solution was evaporated to dryness to produce a pale yellow solid. Crystallisation from methyl alcohol and ether (3:1) produced a crystalline product whose characteristics suggested that it was a sample of ergosteryl B3 acetate.

Product.	Windaus - Ergosteryl B3 Acetate
m.p. 129.5°C	m.p. 132°C
$[\alpha]_D^{18}$ -181.7 $CHCl_3$	$[\alpha]_D^{19}$ -182.9 $CHCl_3$.

App. I, 26. Isomerisation and Deuteration of Cholesterol.

Cholesterol. Alcohol crystallisation produced a starting material, m.p. 149°C. $[\alpha]_D^{20}$ -39.5° ($CHCl_3$).

Water, Acetic Acid, Platinum Black on Asbestos drawn from stocks used previously, App.I, 3 and 5.

Heavy Water. "Norsk Hydro". 99.57% w/w D_2O.

Constituents of Reaction Tube

Catalyst	1.447 gm.
Heavy Water	8.960 gm.
Water	2.675 gm.
Acetic Acid	33.832 gm.
Cholesterol	10.580 gm.

Procedure. The vessel was evacuated and sealed and shaken for three days at 125-129°C. The tube then contained a colourless liquid with a grey oil floating on top and the grey catalyst at the bottom.

Recovery of Acetic Acid. The contents of the tube were washed into a distillation apparatus with a little hot alcohol and the volatile liquids distilled off under reduced pressure. The acetic acid was then converted to silver acetate for deuterium analysis. The residues were dissolved in alcohol and the catalyst filtered off.

Recovery of the Sterols. Attempts at crystallisation from alcohol and benzene alcohol mixtures produced only oils. At this stage an ultraviolet absorption study in alcohol indicated maxima at 226 mμ and 233 mμ fig.8. No isomerisation to sterols containing heteroannular or aromatic ring systems had occurred.

The residues were then chromatographed on "Spences Activated Alumina" which had been dried for several days as 100°C. The column was moistened with freshly distilled carbon tetrachloride and the sterols in carbon tetrachloride solution allowed to pass through. Elution was by carbon tetrachloride (300 ml.) and by 0.1%, 0.2%, 0.5%, 1%, 2%, 5%, 10%, 50%, 100% ethyl alcohol in carbon tetrachloride. 100 ml. of each solvent was used and fractions collected, evaporated to dryness on the steam bath and treated as follows:

Fraction	Crystallisations from	Product	Crystallisations from	Product	Crystallisations from	Product
1	EtOH	oil	MeOH.Bz	Crystals m.64°	MeOH.Bz	
2	EtOH.Et_2O	solid	MeOH.Bz	oil	MeOH.Bz	
3	EtOH	oil	MeOH.Me_2CO	oil		
4	Me_2CO.MeOH	oil	MeOH.Me_2CO	solid	MeOH.Et_2O	crystals m.104°
5	EtOH	oil	MeOH.Et_2O	oil		
6	EtOH	solid	MeOH	solid	Me_2CO	gum
7	MeOH	crystals	MeOH	crystals m.77	MeOH	
8	Me_2CO	crystals	MeOH	crystals m.78		
9	EtOH.Bz	crystals m.72-4	MeOH	crystals m.78		
10	MeOH	crystals	MeOH	crystals m.78		
11	MeOH	crystals	MeOH	crystals m.80		
12	MeOH	crystals	MeOH	crystals m.79		
13	MeOH	solid	MeOH	solid		
14	MeOH	solid	MeOH	crystals m.144	EtOH	crystals m.147.5
15	MeOH	solid/gum				
16	MeOH	trace solid				
17	MeOH	do.				
18	MeOH	do.				
19	MeOH	do.				
20	MeOH	do.				
21	MeOH	do.				
22	MeOH	do.				
23	MeOH	do.				
24	MeOH	do.				
25	MeOH	brown oil				

Examination of Fractions.

Fraction 4. Further crystallisations from ethyl alcohol yielded a product m.p. 114.5°C. $[\alpha]_D^{19}$ -40.5° ($CHCl_3$) This corresponded to the data for cholesteryl acetate.

Fractions 7 to 12. These were of similar crystal appearance (needles) and melted between 77°C and 80°C.

Specific Rotations 9. $[\alpha]_D^{19}$ +82.4° ($CHCl_3$)

8. $[\alpha]_D^{19}$ +86.7° ($CHCl_3$).

Rosenheim Reaction. This test, in which a few crystals of fraction 9 were dissolved in 90% trichloroacetic acid, was negative. This indicated the absence of 3 "α" or 3"β" $\Delta 4$ cholestenols. A test with ergosterol gave an immediate red colour.

U.V. Absorption. An absorption curve for fraction 8 in ether is shown in fig.9.

Attempted Hydrolysis. To exclude the possibility of fraction 9 being an acetate, an attempted hydrolysis was conducted as follows:

.02 gm. potassium hydroxide in 15 ml. alcohol were added to fraction 9 and the solution warmed on the steam bath for 60 minutes. The alcohol solution was diluted with water and extracted with ether. The product, on crystallising from alcohol, had the same melting point as the starting material. The result suggested that fraction 9 was not a steryl acetate.

Preparation of Semicarbazone of Fraction 8.

Diels (41) method was employed.

.3 gm. semicarbazide hydrochloride in 1 ml. water were added to .3 gm. potassium acetate in 3 ml. methyl alcohol. The potassium chloride produced was filtered off. .3 ml. of this solution was added to .06 gm. of fraction 8 producing an immediate gelatinous precipitate. On standing for several days a white powder separated out from the pale yellow mother liquors. This, when washed with methyl alcohol, melted at 210°C. Crystallisations were performed from a 50:50 mixture of methyl and ethyl alcohols.

The product melted at 231°C. (d).

Fraction 14.

Crystallisations from ethyl alcohol yielded a product with the melting point and rotation of cholesterol $[\alpha]_D^{20}$ -40.01° ($CHCl_3$). m.p. 147.5°.

Yields of Purified Products

Cholesterol	.55 gm.
cholesteryl acetate	.21 gm.
cholestenone	.3 gm.

Fig.8. U.V. Absorption Curve. Cholesterol Deuteration. Products in Alcohol.

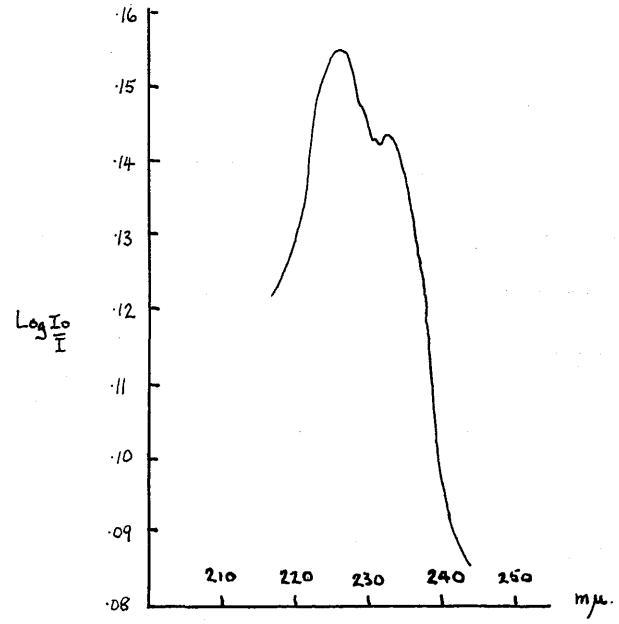
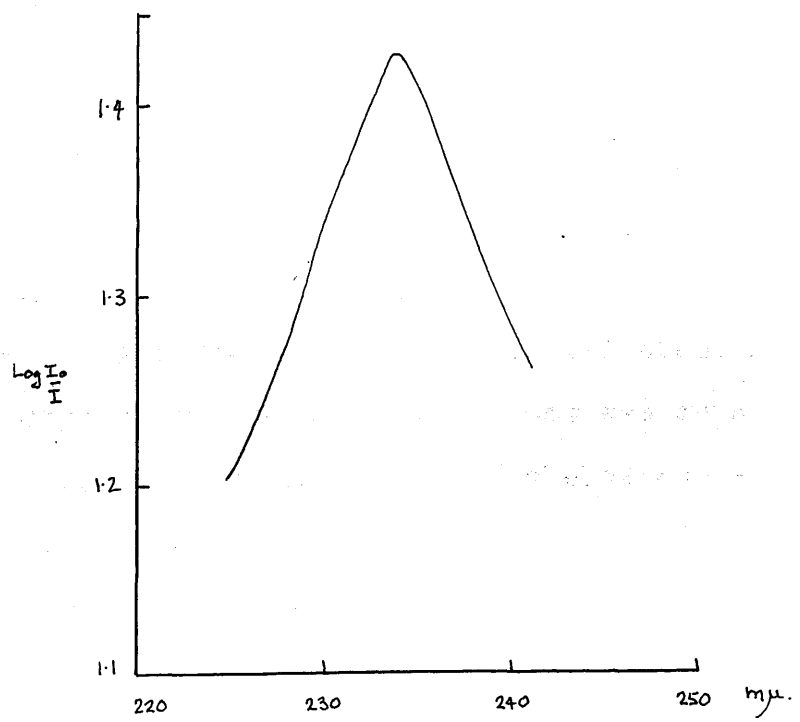


Fig.9. U.V. Absorption Curve. Fraction 8. Cholesterol Deuteration. Solution in Ether.



Conversion Deuterio-cholesterol to Δ 4-cholestenone.

a. Bromination (45). 0.464 gm. deuterio cholesterol was dissolved in 4.6 ml. ether and 2.32 ml. of a solution of bromine in acetic acid added. (2.032 gm. bromine in 21.135 gm. glacial acetic acid). The liquids set almost at once to a solid yellowish mass. This was diluted with water and acetic acid (1:3 by volume). The suspension was filtered, washed with water and the cholesterol dibromide dried in vacuum.

b. Oxidation.(46). 0.65 gm. dibromide was dissolved in 6.5 ml. benzene and 6.5 ml. of a solution of chromium trioxide in aq. acetic acid added (1.2 gm. chromium trioxide in 12 ml. water and 24 ml. glacial acetic acid). The liquids were shaken for six hours at room temperature and the benzene layer separated off and washed with water until neutral to litmus. The benzene solution was dried with sodium sulphate and its volume reduced in vacuum. The residue of 5,6-dibromocholestan-3-one treated as follows.

c. Debromination (47). 0.3 gm. dibromide were dissolved in a mixture of 10 ml ethyl and 10 ml. methyl alcohols. 0.3 gm. zinc dust were added and 0.8 ml. 4 N sulphuric acid a The solution was boiled for ten minutes and filtered. After evaporating to dryness in vacuum, the product was crystallised from methyl alcohol. The product, Δ 4-cholesten-3-one melted at 79.5°C. Yield, 0.11 gm.

App.I. 28. Attempted Deuteration of Cholesterol using heavy water, a platinum catalyst, dilute sulphuric acid in alcohol as a solvent.

Cholesterol. Purification of sterol was accomplished by many crystallisations from alcohol.

Product m.p. 148.5°-149°C. $[\alpha]_D^{20} = -39.5°$.

Heavy Water. 99.57% D_2O, w/w "Norsk Hydro".

Adams' Catalyst. Prepared as in App.I, 3.

Dilute Sulphuric Acid. 2 Normal aqueous acid.

Alcohol. Pure dried alcohol. (21).

Method.

0.393 gm. Adams' Catalyst were weighed into alcohol and hydrogen passed in for one hour to reduce the catalyst to its active form. Nitrogen, to remove as much hydrogen as possible, was passed through the suspension for two hours. The weight of alcohol was then made up to 9.544 gm. 2.211 gm. heavy water, 1.090 gm. dilute sulphuric acid and 2.547 gm. cholesterol were added. This mixture was refluxed on a steam bath for 26 hours after fitting an "anhydrone" guard tube.

After refluxing the mixture was diluted with hot alcohol and the catalyst filtered off.

The filtrate produced crystals on cooling. These were crystallised from alcohol till they melted at 148°C.

App.I, 29. An Attempted Deuteration of Ergosterol with Glacial Deuterio Acetic Acid and a Platinum Catalyst.

Preparation of anhydrous deuterio acetic acid.

Triacetyl borate was prepared from boric acid and acetic anhydride (29). The deuterio silver acetate was prepared by an exchange reaction between acetic acid and heavy water in the presence of a platinum catalyst. (1.04 gm. acid, .55 gm. 99.77% by wt. D_2O, 0.05 gm. platinum black on asbestos catalyst, shaken for several days at 123°C-133°C). The acid was isolated as the silver salt.

Anhydrous deuterio acetic acid.

13 gm. silver acetate were dissolved in 550 ml. water. The solution was boiled and filtered and the salt recovered by evaporation, cooling and collecting of crystals. These were dried at 133° for 17 minutes and for 1 hour at 100°C at 14 mm. pressure.

Phosphoric acid ('Analar') was heated at 100°C at 16-20 mm. pressure for 50 min. 12.3 gm. silver acetate were then placed in a flask and the phosphoric acid added. This mixture was refluxed for 20 min., then distilled. The first few drops were discarded and the remaining acetic acid collected at 115°C-116°C. The product melted at about 2°C. To the 4 ml. of acid was added 2 ml. of glacial acetic acid, m.p. 16.8°C. M.p. of mixture 7°C., i.e., 93.4% acetic acid by weight. To this acid 4.2 gm. of boron triacetate were

added and the mixture refluxed for 4 hours, then distilled, m.p. 16°C., i.e., the product still contained a trace of moisture. 0.7 gm. boron triacetate added and refluxing continued for 4 hours, after distillation m.p. of product was 16°C. The process was therefore repeated once again. On cooling the acid, a thermometer immersed in it fell to 10°C, then rose to 16.8°C till all the acid had solidified. After further cooling the acid was melted and temperature of disappearance of the last crystals was 16.9°C. The volume of the yield was 7½ ml. A few ml. of this acid were converted to silver acetate to await combustion and estimation of the methyl group deuterium content.

Ergosterol, m.p. 163.5°C. $[\alpha]_{Hg}^{20}$-160.2° purified by recrystallisation from an alcohol and benzene mixture (2:1), (20), was dried at 100° for 1 hour at 12-14 mm.

Platinum Black. This was prepared as in App.I, 5.

Reaction Mixture. 3.1650 gm. of the deuterio acetic acid, .6220 gm. catalyst and .7920 gm. ergosterol were weighed into a "Pyrex" tube. The contents were frozen and the tube evacuated and sealed. The tube was shaken for 3 days at 123°C-133°C, then opened after cooling in an acetone, solid carbon dioxide freezing mixture. The contents of the tube were washed into a flask with ether and the ether and acid were distilled off under reduced pressure.

The distillate was added to 7 gm. silver oxide and a few ml. water added. Mixture warmed on steam bath and the silver acetate then recovered by filtrations and crystallisations.

The residue of sterol, etc. was dissolved in ether and filtered free of catalyst. After evaporating off the ether the solid, containing no doubt some ergosteryl acetate, was hydrolysed with a solution of $2\frac{1}{2}$ gm. potassium hydroxide and 2 ml. water in 50 ml. absolute alcohol. The alcohol was removed under vacuum and the moist residue finally dried on the steam bath. This residue was extracted with water and the organic residue dissolved in 80 ml., 40-60° petroleum ether and 10 ml. benzene. This solution was then chromatographed using an aluminium oxide column prepared by drying the oxide for 2 hours at 120-130°C, suspending it in benzene, and then pouring the suspension into a suitable glass column. The column was washed first with 40-60° petroleum ether and the solution then added.

At first a yellow solution was obtained on elution with petroleum ether, yielding a red oil on evaporating to dryness. Secondly a clear solution was produced and this yielded a little white solid. Elution with benzene and then 4:1, alcohol : benzene, yielded yellowish solutions.

All samples yielded small quantities of crystals on recrystallising from alcohol. These were united and re-

crystallised three times from alcohol. Product, white solid m.p. 142°C. in vac. This was recrystallised from 2:1 alcohol : benzene three times and melted at 145°C in vac. Mixed m.p. with ergosterol (m.p. 163.5°) was 158°C. Yield, .0890 gm.

The optical rotation of the substance was then measured and found to be $[\alpha]^{18}_{5450}$ -76.3°, $[\alpha]^{18}_{5850}$ -63.5°, c.f., original sterol $[\alpha]^{20}_{Hg}$ -160.2°.

The low values for melting point and specific rotation indicated that the product was not pure ergosterol.

A further chromatograph of the substance made no change in the melting point of the sterol. The possibility of isomerisation was considered and it seemed likely that this was an impure sample of ergosterol B1 (m.p. 148°C, $[\alpha]_D$ -40°).

App.I, 30. Attempted Deuterations in Solutions containing Components Decomposing by Free Radicle Mechanisms.

Experimental Details.

The chemicals used for these experiments were cyclohexane and acetic acid, purified as in App.I, 5. "Analar" ferrous sulphate, and "Norsk' Hydro" heavy water, 99.7% w/w. The hydrogen peroxide used was "40 volume". The substances were weighed into a flask as follows:

Acetic Acid	1.0265 gm.
Heavy Water	0.5590 gm.
Cyclohexane	2.4610 gm.
Hydrogen Peroxide	.5595 gm.

To these was added .1645 gm. ferrous sulphate and an immediate reaction ensued. The aqueous layer turned brown in colour. The mixture was then maintained at 80°C for 8 hours. After cooling the cyclohexane was separated and dried over caustic soda and the acetic acid recovered by acidifying the aqueous layer with sulphuric acid and steam distillation. The acid was isolated as its silver salt and dried prior to combustion and deuterium analysis.

For the reaction mixture containing the dibenzoyl peroxide, the cyclohexane, acetic acid and heavy water used were as above. The dibenzoyl peroxide was prepared from benzoyl chloride and hydrogen peroxide (30).

The contents of the reaction flask were as follows:-

Acetic Acid	1.1405 gm.
Heavy Water	0.5730 gm.
Cyclohexane	2.3545 gm.
Dibenzoyl Peroxide	.32 gm.

This mixture was warmed for 8 hours at 80°C and then cooled.

The cyclohexane was separated off and recovered by distillation at 30°C-40°C at 3-4 cm. to avoid undue heating of the peroxide. The distillate was dried with caustic soda and distilled. The product was then ready for deuterium analysis.

The aqueous layer containing the acetic acid was added to 30% excess silver oxide and the acid recovered as silver acetate.

Appendix II. Calculations.

App.II, 7. Total Deuterium Atoms Transferred into Hydrocarbons.

Atom % D in n-hexane (Expt. 1) $= \frac{.041 \times 100}{11.11}$ %

No. deuterium atoms in 3.106 gm.

hydrocarbon $= \frac{.04\,1 \times 14 \times 3.\,106 \times N}{11.11 \times 86}$ ...(1

Atom % in Ac' $= \frac{1.371 \times 100}{10.96}$ %

Deuterium transferred into Ac' $= \frac{3 \times 1.635 \times 1.371 \times N}{60 \times 10.96}$ ....(2

Total deuterium transferred = (1 + (2 .

App.II, 11. Calculation made to Discover the Possibility of an Equilibrium Exchange between Octene I and the Solvent.

Total D atoms present in reaction mixture .04325N.

No. removed by acetic acid methyl group .00799N.

No. remaining for distribution .03526N.

No. positions for H or D atoms in water .04325N.

In the acetic acid ionisable hydrogen positions $\frac{1.651N}{60}$

In the hydrocarbon assuming two atoms in equilibrium $\frac{2 \times 3.9765N}{112}$

No. positions assumed to be at equilibrium in octene I = 2.

No. deuterium atoms expected in deuterio octene

$$= \frac{3.9765/56}{\frac{3.9765}{56} + 0.04325 + \frac{1.651}{60}} \times .03526N$$

$$= .01766N$$

Experimental Value ± 0.01133N.

Assuming that 1 atom in each octene I molecule is in equilibrium with the solvents

No. of atoms D expected in octene I = .01178N.

Similarly for 1 atom at equilibrium in the case of pentene I

No. of atoms D expected in pentene I = .01148N

(cf. Experimental, .00980N).

It is concluded therefore that the amount of deuterium introduced into pentene I and octene I is less than that for any "equilibrium" value.

App.II, 13. The Deuteration of Benzene.

The results of experiments 12 and 20 were examined to investigate the possibility of an equilibrium state between the deuterium and hydrogen atoms of the benzene, and the deuterium and hydrogen atoms of the water present and the ionisable acid hydrogen from the acetic acid. It was assumed that the methyl group deuterium atoms of the acetic acid were removed from this system.

In experiment 20 the constituents of the tubes were .0810 gm. catalyst, 2.7900 gm. benzene, .4321 gm. D_2O (99.75 gm. D_2O/100 gm. mixture), 1.647 gm. acetic acid.

Total no. of deuterium atoms present was divided between ionisable hydrogen positions of the acetic acid, the methyl group of the acetic acid, the water present and the benzene.

(1). No. of exchangeable H atoms of acetic acid (ionising H atoms). 60 gm. $H\bar{A}c$ have 1N positions which can undergo exchange. 1.647 gm. have $\frac{1}{60}$ x 1.647N positions.

(2). The methyl group deuterium atoms of acetic acid.

60 gm. $H\bar{A}c$ contain 3N H atoms.

1.647 gm. contain $\frac{3}{60}$ x 1.647 x N atoms.

The heavy water produced by burning the silver acetate contained 3.02 gm. D_2O : 96.98 gm. H_2O

i.e., $\frac{4}{20}$ x 3.02 gm. D : $\frac{2}{18}$ x 96.98 gm. H

.302 D atoms : 10.74 H atoms.

∴ fraction of methyl group atoms which were D atoms

$$= \frac{.302}{.302 + 10.74}$$

∴ No. of D atoms in $A\bar{c}'$ = $\frac{3}{60}$ x 1.647 x N x $\frac{.302}{.302 + 10.74}$

Now total D atoms added to reaction mixture (as D_2O)

= .4321 x $\frac{4}{20}$ x $\frac{1}{2}$ N atoms

Total D atoms distributed between positions available in water etc. will be

(total deuterium atoms added) - (deuterium atoms in methyl group) = .04321N - $\frac{1}{20}$ x 1.647 x $\frac{.302}{11.042}$ N

Now no. of positions available in water added = .04321N.

Let No. of positions for substitution in benzene be six per molecule,

Then 78 gm. have 6N positions for deuteration,

2.7900 have $\frac{6N}{78}$ x 2.7900.

Assuming an even distribution of H and D atoms, fraction of D atoms in benzene will be

$$\frac{\frac{6N}{78} \times 2.79}{\frac{6N}{78} \times 2.79 + .04321N + \frac{1}{60} \times 1.647N}$$

and ∴ no. present in benzene will be this fraction x total available deuterium atoms.

$$= \frac{\frac{6N \times 2.79}{78}}{\frac{6N}{78} \times 2.79 + .04321N + \frac{1.647N}{60}} \times \left[.04321N - \frac{1.647 \times .302N}{20 \times 11.042} \right]$$

$$= R.$$

$$\text{Now atom \% D in benzene} = \frac{\text{total D atoms in benzene}}{\text{total D + H atoms in benzene}}$$

$$= \frac{R}{\frac{6N \times 2.79}{78}}$$

$$= \underline{14.35 \text{ atom \%}}.$$

<u>Experimental value found = 12.75 atom %.</u>

It was therefore concluded that the benzene had six hydrogen positions in equilibrium with the heavy water.

<u>App.II, 17. Mechanism I. Isomerisation and Deuteration of Cyclohexene.</u>

$$3C_6H_{10} = 1C_6H_6 + 2C_6H_{12}$$

The results of Expt.2 showed that the cyclohexane, when deuterated alone with acetic acid, showed a D_2O % of .31 in the analysis. We will assume for an approximate calculation therefore, that only the benzene and acetic acid were deuterated in experiment no.5.

Constituents of tube Expt.5.

D_2O .4315 gm.

$HA\bar{c}$ 1.648 gm.

⬡ 2.9540 gm.

Catalyst .0810 gm.

Weight of benzene produced = $\frac{78 \times 2.954}{3 \times 82}$ gm.

" " cyclohexane produced = $\frac{2 \times 84 \times 2.9540}{3 \times 82}$ gm.

No. of H or D positions for weights of substance

D_2O .04315N

$HA\bar{c}$ 1). Carboxyl group $1.648 \times \frac{1}{60}N$

2). Methyl group $1.648 \times \frac{3}{60}N$

Benzene $\frac{78 \times 2.954 \times 6}{3 \times 83 \times 78} N = \frac{2 \times 2.954}{82} N$

Cyclohexane $\frac{2 \times 84 \times 2.9540 \times 12}{3 \times 82 \times 84} N = \frac{8 \times 2.9540}{82} N$

Experimental evidence showed that methyl group of acetic acid produced 5.33% D_2O w/w, i.e., $\frac{.533 \times 100}{.533 + 10.52}$ atom %

∴ for $\frac{1.648}{20}N$ positions, no. of deuterium atoms present would be $\frac{1.648}{20}N \times \frac{.533}{.533 + 10.52}$

i.e., this No. D atoms removed from system in methyl group.

Now total D atoms in tube = .04315N

∴ remaining D atoms left for distribution in benzene, water, etc. = $.04315N - .0824N \times \frac{.533}{.533 + 10.52}$

Total no. of positions in which this deuterium is distributed

$$= \frac{1.648}{60}N + .04315N + \frac{2 \times 2.954}{82}N$$

Assuming even D distribution, atom % D in benzene will be

$$\frac{100 \times \dfrac{\dfrac{2 \times 2.954}{82}N}{.02747N + .04315N + \dfrac{2 \times 2.954}{82}N} \times \left[.04315N - .0824N \times \dfrac{.533}{11.05}\right]}{\dfrac{2 \times 2.954 \times N}{82}}$$

Taking H atoms produced from combustion cyclohexane into account, atom % D in product

$$= \frac{\text{total D atoms in benzene}}{\text{total D + H atoms in benzene + total H atoms in cyclohexane}}$$

$$= \frac{\dfrac{\dfrac{2 \times 2.954 \times N}{82}}{.02747N + .04315N + \dfrac{2 \times 2.954}{82}} \times \left[.04315N - \dfrac{.0824N \times .533}{11.05}\right] \times 100}{\dfrac{10 \times 2.954 \times N}{82}}$$

$$= \underline{5.492 \text{ atom \%}}.$$

Experimental value = 8.337 atom %.

This examination was unsatisfactory and led to the consideration of Mechanism II.

App. II, 17.

$$\text{Atom \% D at start of reaction} = \frac{.04315 \times 100}{.04315 + .02747}$$

$$= \underline{61.2\%}.$$

Atom % D at end of reaction

$$= \frac{100 \times \left[\underset{(D_2O)}{.04315} - \underset{\text{(methyl group)}}{.0824 \times \frac{.533}{11.05}} - \underset{\text{(product)}}{\frac{8.337}{100} \times \frac{10}{82} \times 2.954}\right]}{.04315 + .02747}$$

$$= \underline{12.91\%}.$$

Average value during deuteration = 37.05 atom %.

If two molecules of this average mixture are now introduced into cyclohexene to produce cyclohexane, then

Atom % D in cyclohexane $= \frac{2 \times .371 \times 100}{12} = 6.18\ \%.$

If benzene attains equilibrium with the mixture of heavy water at the end of the reaction

No. D atoms in one molecule = 6 x .129

No. D atoms in one molecule cyclohexane = 2 x .371

Atom % in products (2 C_6H_{12} : 1 C_6H_6)

$$= \frac{(2 \times 2 \times .371) + 6 \times .129}{30} \times 100\ \%$$

$$= 7.53\ \%.$$

Experimental Value, 8.34 %.

App.II, 19. Calculation on Expt. 18. The Deuteration of 1:4 Cyclohexadiene.

(1). Assuming that the deuterium content of the product (benzene plus cyclohexane) corresponded to 8.32 atom % and that the atom% deuterium in the cyclohexane was 11.70, the following calculation was made to determine the atom % deuterium to be expected in the benzene.

$$3\ C_6H_8 = 2\ C_6H_6 + C_6H_{12}$$

3 x 80 gm. 2 x 78 gm. 1 x 84 gm.

Number of positions in 2.853 gm. cyclohexadiene for D or H atoms $= \frac{2.853 \times 8 \times N}{80}$

Number of positions in benzene and cyclohexane will be respectively

$$\frac{2 \times 78 \times 2.853 \times 6N}{3 \times 80 \times 78}, \qquad \frac{84 \times 2.853 \times 12 \times N}{3 \times 80 \times 84}$$

i.e., 0.2853N positions in cyclohexadiene produce 0.1427N + 0.1427N positions in the products.

Atom % D in water from product (benzene and cyclohexane)

$$= \frac{0.916 \times 100}{11.01}$$

$$\text{Number of D atoms in product} = \frac{0.916 \times 0.2853 \times N}{11.01}$$

$$\text{Atom \% D in cyclohexane} = \frac{1.28 \times 100}{10.97}$$

$$\text{D atoms in cyclohexane} = \frac{1.28 \times 0.1427N}{10.97}$$

Atom % to be expected in the benzene

$$= \frac{\frac{0.916 \times 0.2853}{11.01} - \frac{1.28 \times 0.1427}{10.97}}{0.1427} \times 100 = \underline{4.97\%}$$

Found ... $\underline{3.82\%}$

(2). Assuming that the isomerisation of the cyclohexadiene takes place followed by an exchange between the benzene and the heavy water, a calculation was made of the "equilibrium" value for the deuterium content of the benzene. It was assumed that six atoms per molecule attained equilibrium.

Equilibrium value for Benzene.

Total D atoms in tube $= .0431N$

$$\text{D atoms in cyclohexane} = \frac{1.28 \times .1427 \times N}{10.97}$$

$$\text{D atoms in methyl group of acetic acid} = \frac{1 \times 1.643 \times .466 \times N}{20 \times (.466 + 10.59)}$$

D atoms left for distribution in benzene, water, carboxyl hydrogen = (.0431 - .01664 - .003461)N = .0230N.

Positions for even distribution of D atoms

$$= (.0431 + .1427 + \frac{1.643}{60}) \times N$$

Atom % D in benzene = $\frac{.0230 \times 100}{.2132}$ = 10.80%.

(3). If into a cyclohexadiene molecule we introduce four atoms from the solvent at the beginning of the isomerisation these are more likely to be deuterium atoms. As the reaction proceeds the heavy water is diluted and hydrogen introduction becomes more likely. This theory makes the tacit assumption that each molecule undergoes exchange once only during the course of the reaction.

Calculation of atom % D in solvent at end of reaction -

$$\frac{(.0431 - .0035 - \frac{.916 \times .2853}{11.01}) \times 100}{.0431 + .0274}$$

(D_2O) (CH_3) (product) (water) (COOH)

$$= \frac{(.0431 - .0035 - .02373) \times 100}{.0705}$$

$$= \underline{22.55\%}$$

Atom % at start - $\frac{.0431 \times 100}{.0431 + .0274}$ = $\underline{61.2\%}$

Average value = $\underline{41.9\%}$.

If four D atoms from this average mixture are now introduced into a molecule of cyclohexadiene to produce cyclohexane we can calculate the atom % in the product .

$$\text{Atom } \% = \frac{.419 \times 4 \times 100}{12} = 13.9\%. \qquad \text{Found, } 11.70\%.$$

If the benzene is also at equilibrium with the heavy water at the end of the disproportionation -

No. D atoms in one molecule benzene = 6 x .226

" " " " " " cyclohexane = 4 x .419

Atom % in product (2 benzene : 1 cyclohexane)

$$= \frac{(2 \times 6 \times .226) + 4 \times .419}{24} \times 100$$

$$= 18.3\%.$$

It is concluded that the benzene exchange did not attain the state described in the calculations.

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Additional Paper

on

The Combustion of Sulphur to

Sulphur Dioxide.

I N D E X

Introduction.

The relative abundance of the isotopes of sulphur may be determined by mass spectrometric analysis of sulphur dioxide. In connection with experiments on enrichment of S^{34} sulphur, a conversion process from sulphur to the dioxide was required. This process, besides giving maximum yields, was to be suitable for conversion of some 8 mg. sulphur: contamination of the product with substances in the mass range 64 to 72 had also to be avoided.

Discussion on Methods.

In the combustion of sulphur to sulphur dioxide, the possibility of using an inhibitor to minimise production of sulphur trioxide was suggested by the work of Keyes (1), who stated that phenolic bodies apparently inhibited the further oxidation of sulphur dioxide. Phenol was chosen therefore and its "inhibiting" effects were investigated.

Norrish and Rideal (2) reported that, in the combustion of sulphur in air, the formation of sulphur trioxide became apparent above 305°C, but combustions attempted at this temperature were found to be slow. At 460°C the burning of sulphur in air or oxygen, in the absence of liquid sulphur, produced about 3.6% sulphur trioxide, according to Cornog, Dargan and Bender (3).

In the experiments to be described, several combustions were conducted at a temperature, between the two mentioned, of

350 to 360°C. In the cooler parts of the combustion tube was placed a boat containing phenol in such a position that it was just molten. The air for combustion was passed over this phenol and the sulphur was burned in the presence of the vapour. Preliminary attempts at measuring the quantity of sulphur dioxide produced by passing the effluent gases into standard iodine solution and back titrating the excess with thiosulphate were unsatisfactory.

Reproducible results could not be obtained and a change to potassium permanganate as absorbing solution was also ineffective.

A new method of estimating sulphites and then sulphur dioxide gas was therefore evolved; this was found to be satisfactory in the presence of arbitrary quantities of air and phenol. Preliminary investigations thereon were made by estimation of sodium sulphite solution and comparing results with those from the usual iodine and thiosulphate methods.

An Estimation of Sodium Sulphite.

The method was based on the known fact that when aqueous solutions of a sulphite, bromate and silver nitrate were mixed, the bromide ions produced by the reduction of the bromate were precipitated as silver bromide. This reaction was investigated on a quantitative basis and it was found that the weights of silver bromide produced gave an accurate estimation of the

concentration of the parent sulphite solutions. Further, the method was satisfactory in the presence of arbitrary quantities of air and phenol and results compared well with those obtained by concurrent estimations by iodine and thiosulphate.

Experimental Details.

A standardised procedure was adopted. "Analar" sodium sulphite crystals were used to make aqueous solutions of strength about 1%. The solutions were used immediately on preparation. Several portions were pipetted into standard iodine for routine analysis and others into a potassium bromate-silver nitrate stock solution, from which silver bromide precipitated. In the preparation of the bromate-nitrate solution, 6 gm. of each were dissolved in 500 ml. water, the solutions mixed and diluted to 1700 ml. The suspension was warmed and cooled. 340 ml. of dilute sulphuric acid was then added and the suspension filtered. Just before adding the sulphite solution, 50 ml. of this solution were diluted with 25 ml. distilled water and the solution filtered through an asbestos pad. If the sulphite solution was run into a solution of bromate-silver nitrate, acidified with dilute sulphuric acid, then precipitation was complete, whereas in neutral solution the precipitate obtained was soluble in excess sulphite solution.

Phenol, 0.05 to 0.1 gm., was added and the sulphite solution then run in. A fine white suspension of silver

bromide was produced: this was heated for 15 to 20 minutes in a steam oven and then cooled rapidly in tap water. A few drops of concentrated nitric acid were added to reduce the solubility of the silver bromide. The precipitate coagulated on stirring and was collected by Pregl's suction filtration method. The precipitate was loosened from the beaker by successive quantities of dilute nitric acid and rectified spirits, and quantitative transfer to the crucible accomplished. The final washing of precipitates in the crucibles was done with 25 ml. dilute nitric acid (1 ml. conc. in 100 ml. water) and then 25 ml. rectified spirits.

The crucibles, with their asbestos pads, weighed only 3.5 gm. After cleaning with chromic acid, washing with warm water, the final washing was done with 25 ml. dilute nitric acid and 25 ml. of rectified spirits.

The routine for drying and weighing of crucibles was as follows. Drying of crucibles or crucibles with precipitates was done for 30 minutes at 123°C. They were allowed to cool for 5 minutes, wiped with a moist cloth then with a dry cloth, and placed near the balance case. Weighing with calibrated weights was done after 20 minutes.

Results.

"Sulphite" Solution gm/20 ml.	Weight AgBr gm.	Percentage Purity	Percentage Purity by Iodine Thiosulphate
0.2024	0.04522 0.04551	89.6 90.04	90.0
0.1906	0.04224	89.3	90.6
0.2122	0.04756	90.0	88.3
Same solution after 2 hours	0.04385	81.5	79.6

The Combustion of Sulphur in Oxygen and Estimation of the Sulphur Dioxide produced.

The method of estimating sulphites was extended to the estimation of sulphur dioxide produced by burning sulphur in a 12" x $\frac{3}{4}$" combustion tube. Effluent gases were passed into the bromate-nitrate stock solution and the silver bromide produced was weighed and percentage sulphur accounted for as sulphur dioxide calculated. Combustions were done in oxygen with and without phenol vapour.

Reproducible results were obtained which did not vary with quantity of phenol or oxygen used, for "combustions" done with phenol and air only and no sulphur produced no precipitates in the absorbing solutions.

It was found that the presence of phenol in the absorbing solutions was necessary, otherwise low results which could not be reproduced were obtained.

Experimental Details.

The combustion tube, after drying overnight at 120°C, was fitted into place and "flamed out" while a slow stream of pure dry oxygen was passed through the tube. Gas bubblers containing 40 ml. each of the stock bromate-nitrate solution were placed in position, each containing 0.1 gm. phenol in solution. A weighed capsule of sulphur with a pinpoint opening was inserted into position and after "flaming out"

once again, the combustion was started. The furnace was raised rapidly to 500°C and at 440°C the sulphur burned with a bright blue flame. Rate of flow of oxygen was 40 cc. per minute. The sulphur dioxide produced was swept in its entirety into the absorbing solutions and a fine yellow precipitate of silver bromide was produced. This was treated as in the 'Estimation of Sodium Sulphite', and the weight of silver bromide was taken as a measure of the sulphur dioxide produced. No precipitates were obtained in the second bubbler and absorption was therefore complete in the first.

Experiments were then conducted in which sulphur was burned in oxygen which had been passed over molten phenol and the yields of sulphur idoxide noted.

Results.

Combustion of sulphur in oxygen at 440°C.

Combustion Tube	Absorbing Solution	Percentage Sulphur accounted for as SO_2
Phenol absent	Phenol absent	41.5, 34.5
Phenol absent	Phenol present	92.3, 94.2, 93.2
Phenol present	Phenol present	90.06, 90.0

Conclusions.

1. In the combustion of sulphur in oxygen at 440°C, 93% of the sulphur has been accounted for as sulphur dioxide.

2. A gravimetric method of estimating sulphur dioxide and sulphite solutions has been developed.

3. Phenol did not act as an inhibitor in the combustion process and increase the yields obtained of sulphur dioxide. It did however have an inhibiting effect in the estimating process used and thus allowed an accurate estimate of the dioxide produced. The apparent yield of dioxide was thereby increased. The phenol present possibly reacts with sulphur trioxide to produce phenol sulphonic acids.

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